ACUTE CARDIOTOXICITY OF DOXORUBICIN IN RATS

THESIS

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RAKESH KUMAR

C E R T I F I C A T E

This is to certify that the work in connection with the thesis entitled "ACUTE CARDIOTOXICITY OF DOXORUBICIN IN RATS" was carried out in the Department of Pathology under our guidance and supervision by Dr. Rakesh Kumar. The techniques and observations embodied in this thesis have been undertaken by the candidate himself and checked by us .

He has also complied with the necessary stay condition in the Department as required by the regulation of Bundelkhand University, Jhansi.

(SUSHMA GUPTA)
M.D., MNAMS
Associate Professor
Department of Physiology,
M.L.B.Medical College,
Jhansi;
(CO GUIDE)

(R.K.GUPTA)
M.D., MNAMS
Professor and Head
Department of Pathology,
M.L.B.Medical College,
Jhansi
(GUIDE)

Dated: 8th August, 1991

D E D I C A T E D T O

M Y M O T H E R

Who for the last eight years, after the demise of my father is still waiting all alone for me to join her.

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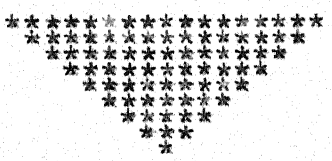


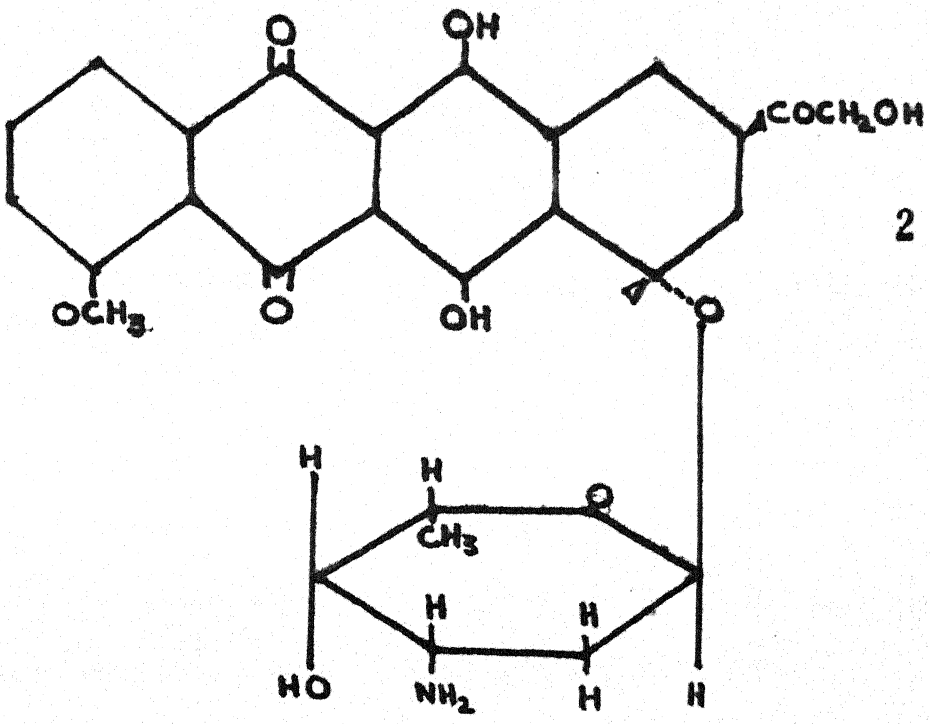
I N T R O D U C T I O N

INTRODUCTION

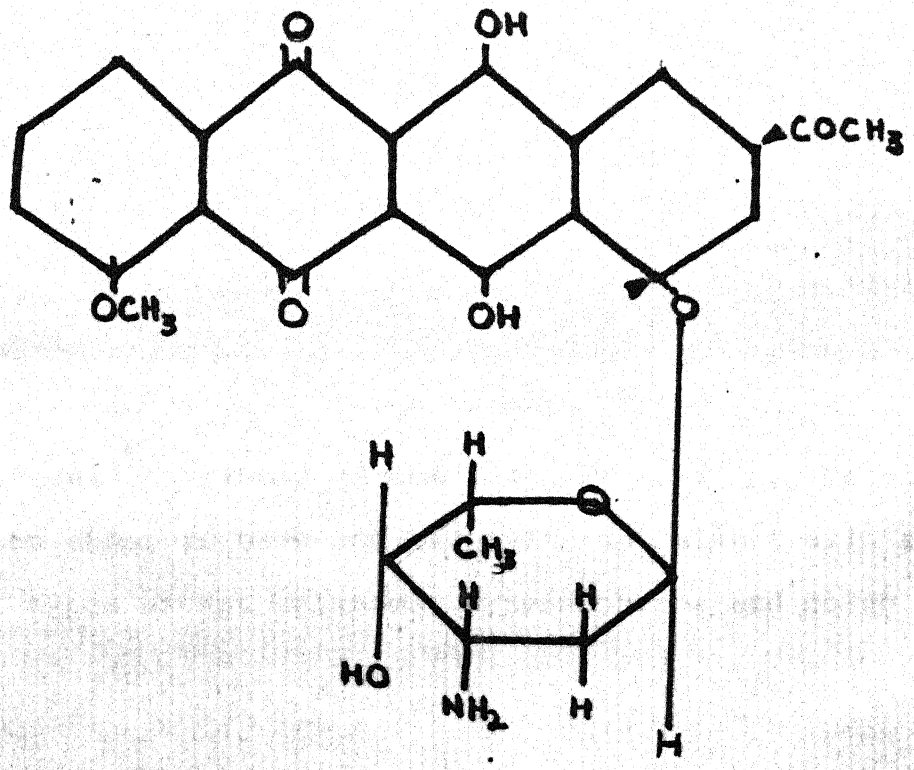
Decades have gone by, still millions are engulfed by deadly tentacles of the cancer. Research and experiments are in progress to limit the genetic change whether spontaneous, or induced by chemicals or viruses that converts a normal cell to an invasive malignant cell. Chemotherapy is one important mode in cancer cure. Antibiotics like actinomycins (dactinomycin), bleomycin, daunorubicin (rubidomycin) and doxorubicin (adriamycin) have justified their use as important anti cancer agents.

Anthracycline antibiotics, is an effective group of chemotherapeutic agents for use against a wide range of solid and haematological malignancies.

Grein et al (1963) isolated an antibiotic from cultures of a mutant streptomyces pencetuis (Streptomyces percitus varcaesive) which was named adriamycin. Its structural formulae is very similar to that of daunomycin from which it differs only in the substitution of a hydrogen atom with a hydroxyl group on the acetyl redical.



DOXORUBICIN



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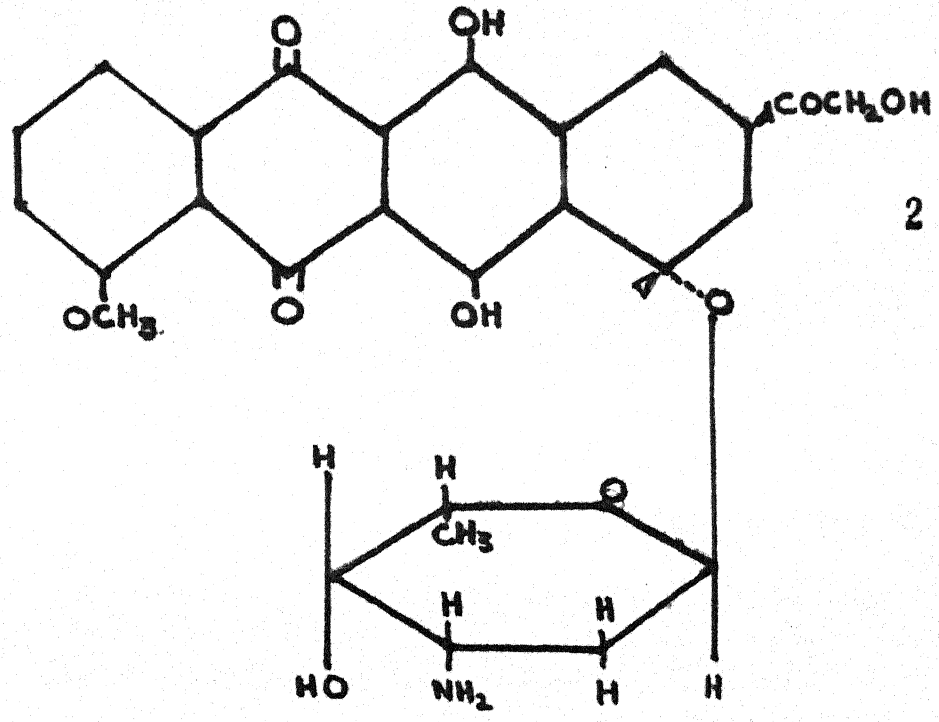

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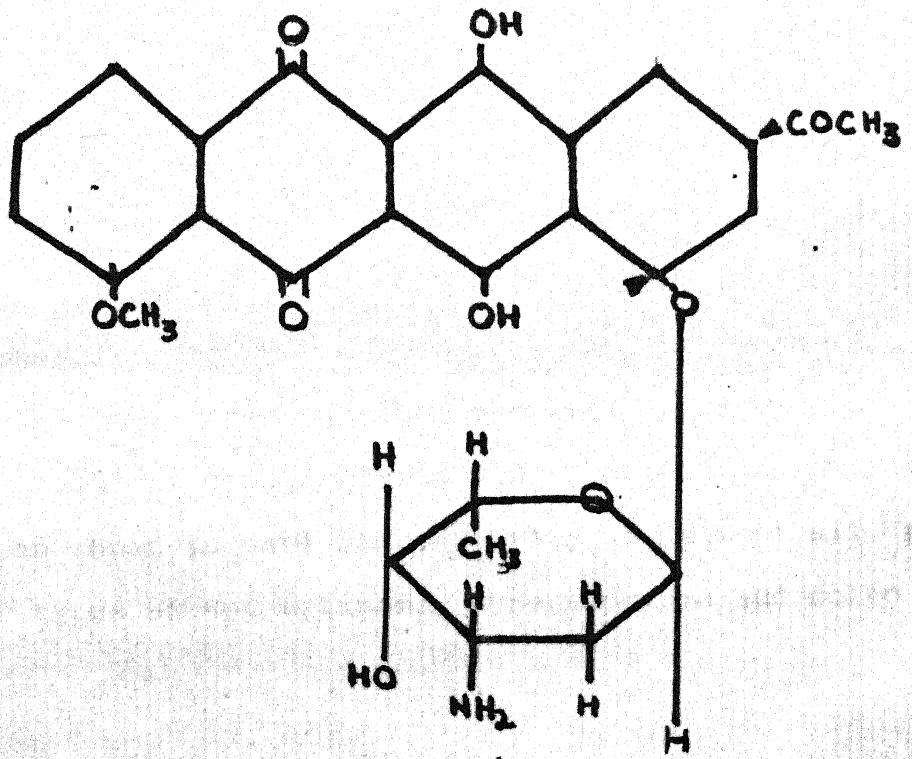

DAUNORUBICIN

In acid media adriamycin is split into two components, a red pigmented water insoluble aglycone (adriamycinone), and a water soluble base reducing amino sugar (daunosamine). Adriamycin belongs to the anthracycline group, as do daunomycin and rubidomycin (daunorubicin).

The preliminary pharmacological studies were carried out by Di Marco et al (1969) at the National Cancer Institute Milan. Comparative studies showed that at equitoxic dose (2 and 2.5 mg) of adriamycin per Kg and 2.6 - 3.25 mg of daunomycin per Kg, the survi al time in C 3H mice bearing lymphosarcoma was higher in animal given adriamycin, while the inhibition dose (ID 50) was found to be 1-5 and 3.3 mg/kg for adriamycin and daunomycin respectively. The conclusion from these preliminary pharmacological studies is that, adriamycin has a higher therepeutic index 1.25 than daunomycin (.67).

Doxorubicin is a principal agent in the treatment of Leukaemias, Hodgkin's and non-Hodgkin's lymphomas, osteogenic sarcoma, breast cancer, ovarian carcinoma, gastric cancer and oat cell carcinoma of the lung. In addition adriamycin has been shown to have some activity in almost all adult tumour types except melanoma, hypernephroma and colon cancer.

Di Marco et al (1965) have demonstrated that mitotic activity and thymidine incorporation are reduced by anthracyclines and that RNA synthesis is inhibited. Subsequent studies have shown that both daunomycin and adriamycin complex with nuclear DNA by intercalating between base pairs, thus causing steric obstruction to DNA dependent RNA synthesis. These properties apparantely explain the molecular basis of the in vitro and in vivo cytotoxic and antimitotic activity exhibited by these antitumour agents against neoplastic and other rapidly dividing cells.

Aside from its promising chemotherapeutic potential, adriamycin have exhibited undesirable side effects which includes severe bone marrow suppression, alopecia, and oral ulcerations. The most unpredictable and unmanageable toxic manifestation, however, is an insidious cardiomyopathy affecting a variable but significant number of treated cancer patients.

There are two distinctly different types of cardiotoxicity associated with the use of adriamycin. First, acute arrhythmias, non-specific ST-T wave changes, and even clinically inapparent decreases in left ventricular ejection fractions have been observed. These acute effects are always transient usually asymptomatic, and do require

modifications of the dose or schedule of administration rarely and that too mostly in patients with prior history of arrhythimas. The second and more serious cardiotoxicity, is the late cardiomyopathy clinically indistinguishable from idiopathic or nutritional cardiomyopathies. The congestive heart failure seen may be insidious in onset but frequently occurs abruptly as pulmonary edema precipitated by intravenous hydration associated with cancer therapy.

Heart failure usually develops within two months of the last dose of drug, but instances of cardiomyopathy developing six months to almost a year later have been reported.

Histologically the cardiac lesions induced by anthracyclines are not distinctly different from those associated with many other cardiomyopathies, but they can be readily distinguished from lesions resulting from radiotherapy or arterial occlusions. Initially the damage is focal and scattered throughout the heart. With larger doses of adriamycin, the heart is diffusely involved. The most frequent manifestations of anthracycline damage are myofibrillar lysis and cytoplasmic vacuolization. Fibrosis as typically seen after radiation damages occurs much less frequently, and there is no inflammatory response.

The pathophysiology of this cardiotoxicity is poorly understood. The hearts of rabbits treated with doxorubicin have abnormally high tissue levels of calcium and it has been proposed that adriamycin induced heart changes are due to a calcium mediated necrosis. However, attempts at preventing the cardiotoxicity using chelating agent in animal models have been unsuccessful. Adriamycin has a glycosidic structure and specifically inhibits sodium potassium activated Atpase. This suggests that the doxorubicin effect might occur at the same site as digitalis toxicity or that a common receptor for the anthracyclines and the cardiac glycoside might be necessary for uptake of these drugs into the heart. Currently available data suggests that strophanthin will not block doxorubicin uptake in animal models and there are insufficient data to justify clinical trials using pretreatment with digitalis compounds. In a recently reported study of Bristow et al (1978) on doxorubicin cardiotoxicity in rabbits, alongwith histamine and catecholamine blocking agents used together seemed to prevent the appearance of a cardiomyopathy. This suggests another possible mechanism that might explain both the acute and chronic effects of doxorubicin on the heart.

Folkers et al (1977) in their study concluded that doxorubicin also inhibits Co- Q10 (ubiquinone) enzyme systems in isolated mitochondria and the administration of ubiquinone to rabbits or rats has been found to at least partially block some of doxorubicins cardiotoxicity, Handerson et al (1980) reported that doxorubicin cardiotoxicity, and possibly even the anthracycline antitumour effect is mediated either through the production of superoxides or by activation of the anthracyclines to a free radical state. It has also been reported by them that lipid peroxidation and histologic changes in murine heart muscle are prevented by pre treatment with alpha-tocopherol, another free radical scavanger.

The study is designed to evaluate the acute effects of the drug on rats heart that may be the first manifestation of a process that ultimately leads to myocyte damage and cardiomyopathy. It is possible that a complete characterization of this process will provide some clue to the pathogenesis, as well as providing a potential model for the evaluation of protective strategies.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The dose limiting toxicities of anticancer drugs are usually confined to tissues with a high mitotic rate such as bone marrow, gastrointestinal mucosa, and hair follicles. The anthracycline antibiotics, daunomycin and doxorubicin (Adriamycin) are exceptions to this rule.Other antineoplastic agents have been reported to cause mild chest pain and E.C.G. changes (5 FU) or acute mild pericarditis when used in very large single doses (cyclophosphamide). Only the anthracyclines consistently induce alteration in cardiac function which limits the safe cumulative dose of drug which can be given. Both the agents exhibit antineoplastic activity against a wide variety of tumours but unfortunately there is development of acute and chronic cardiac damage often interfering with the full therapeutic potential of the drug. The acute form of cardiac toxicity is generally mild and manifests by arrhythmias and electrocardiographic changes. This contrasts with severe, cumulative dose dependant cardiomyopathy following chronic administration of the drug.

In 1969, Herman et al at the National Cancer Institute, Bethesda, Maryland carried out experiments on three rodent species, rat, guineapig and hamsters to study the comparative cardiac toxicity of Daunomycin. The authors

knowing that clinically, total doses greater than 25 mg/kg have been associated with the development of cardiopulmonary symptoms such as tachycardia, with or without arrhythmia, gallop rhythm, congestive heart failure, tachypnea and cases of dyspnea. The studies were initiated to determine whether daunomycin could induce similar or other types of cardiac toxicity in animals. Three male rats (250-300 gms), 5 male guineapigs (450 gms) and 50 male golden hamsters (90-130gms) were given daunomycin in the dose of 50 mg/kg. The cardiovascular system of the rat was relatively resistant to daunomycin during the acute phase of the study. A total of 200-300 mg/kg produced slight decreases in blood pressure and heart rate and some variable changes in the amplitude of E.C.G. waves. In the guineapigs, a total of 75-175 mg/kg daunomycin was given to these animals. After most injections a transient hypotensive effect was seen. Daunomycin had a striking respiratory depressant effect on all animals. Periods of apnoea of 2-4 minutes duration occured following certain doses. Usually any changes in the E.C.G. were secondary to these periods of apnoea. In case of hamsters, in contrast to the rat and guineapig, a slight to moderate pressor response was usually observed during infusion with daunomycin. In addition, alterations in the E.C.G. were usually seen within 10-40 seconds followed by a definite cardiac arrhythmia 20-90

seconds later. The study concludes that cardiac toxicity induced by daunomycin in the hamsters differs from that observed clinically because it is acute and appears predominately as an arrhythmia.

In the year 1973, Chalcroft et al, studied fine structural changes in rat myocardium induced by daunorubicin. Rats were injected intravenously with daunorubicin in the dose of 25 mg/kg body weight to determine the morphologic basis of its cardiotoxic effects. Although prominent changes were not observed by light microscopy in either atrial or ventricular myocardium, significant fine structural changes were seen by electron microscopy in both. One day after injection, daunorubicin treated rats showed membranous whorls inside the mitochondria and in relation to mitochondrial surfaces. While the nucleoli were of normal shape, the nucleolonema appeared more prominent in same muscle cells. Atrial myocardial cells after the same interval showed sacculation of some mitochondrial cristae. Three days after the treatment mitochondrial swelling, decreased matrix density and loss of cristae were evident in some cardiac muscle cells. Five days after the drug infusion both atrial and ventricular muscle cells contained large numbers of degenerating mitochondria. The study shows that striking abnormalities affected progressively more mitochondria of both atrial

and ventricular muscle cells. In the only comparable study of fine structural changes of daunorubicin on myocardium, Liss and Cotton (1971) briefly describe similar intra mitochondrial vacuolation, and also Z line distortion and sarcomeric disorganization but do not refer to mitochondrial membrane changes or capillary endothelial swelling.

Rogers Jaenke et al (1974) worked for the anthracycline antibiotic induced cardiomyopathy in rabbits. The authors found delayed cardiomyopathy similar to that observed in cancer patients during chemotherapy with the anthracycline antibiotics, daunomycin and adriamycin in the rabbit. In chronically treated rabbits, this syndrome was characterized by congestive myocardial failure and focal to disseminated myofibre degeneration and necrosis. Alterations in myocardial cells were characterized by expansion of intracellular membrane bound compartments, the selective isolation and degradation of degenerating mitochondria accompanied by myofibrillar breakdown and eventual complete myolysis and fibrosis. A group of 12 male and 12 female Newzealand white rabbits weighing 2.2 to 3.7 kgs received daunomycin or adriamycin in the doses ranging from 2.4 to 3.0 mg/kg/week. The animals were examined at intervals ranging from 3 to 18 weeks. The distribution of animals and the summary of the relationship of myocardial lesions to total drug received and time adminis - tered are briefly given as follows :-

Drug	No.of Rabbits	Days treated	Total drug received (mg/sq m)	Myocardial alterations
Daunomycin	5	28	100-130	Negative
	4	61-71	200-400	Negative
	3	96	350-450	2/3 focal myofibre degeneration only.
	4	112-126	375-600	1/4 focal myofibre degeneration only.
				1/2 severe myofibre degeneration and necrosis.
				1/2 rabbits with degeneration and necrosis, congestive myocardial failure.
Adriamycin	4	23-30	70-130	Negative
		67-71	250-340	3/4 severe myofibre degeneration and necrosis.
				2/3 rabbits with degeneration and necrosis,congestive myocardial failure.

The ultrastructural study indicates that myocardial degeneration and necrosis were characterized by extensive subcellular alterations following the chronic administration of daunomycin or adriamycin. Although lesions were frequently focal in distribution, some experimental animals developed congestive myocardial failure, indicating that the myocardiopathy could be progressive and fatal in rabbit.

Mettler et al (1977) conducted experiments to study the adriamycin induced cardiotoxicity (Cardiomyopathy and congestive heart failure) in rats. Adriamycin (ADR) was administered to rats at doses of 1 to 2 mg/kg/week for 10 to 14 weeks. The majority of adriamycin treated rats developed cardiomyopathy from 3 to 23 weeks after the last injection. Forty to seventy percent of those rats with cardiomyopathy had gross evidence of congestive heart failure (pleural effusions, ascitis, hepatomegaly, cardiomegaly). Histopathological evaluation of hearts from rats revealed varying degrees of myocardial alterations. The most consistent and predominant finding was marked vacuolization of myocytes, especially in subepicardial and subendocardial regions in addition to perivascular locations. Although vacuolar lesions were observed frequently in ventricles and septa, several rats had severe vacuolation of trial myocytes. In addition, vacuolar degeneration of ganglion cells was observed in two or three rats where atrial sections were found to include clusters of cardiac neurons. Three rats had focal atrial thrombosis evident histopathologically. Myocytolysis, interstitial edema, and mild fibrosis were evident in occasional areas of more severely affected rat hearts. The incidence and severity of the cardiomyopathy appeared to be dose dependent.

Ultrastructural evaluation of samples of myocardium from adriamycin treated rats revealed varying degrees of sarcoplasmic vacuolation. The vacuoles occured predominantly in perinuclear position and consisted of distensions of the sarcoplasmic reticulum, the T-tubule system, and Golgi vesicles. In myocytes where vacuolation was severe, there was a concomitant reduction in the quantity of myofibres. A few scattered myocytes were found to contain fragmented sarcomeres with disruption of the characteristic parallel arrays of myofilaments. In general, mitochondria did not appear to be altered markedly, although several were swollen with disruption of cristae. Formation of myelin figures was not a predominant feature of mitochondrial alterations.

Olson et al (1977) at the Department of Veterinary Pathobiology, College of Veterinary Medicine, Theohio State University, Columbus, Ohio carried out an extensive experimental study to observe cardiotoxicity of adriamycin in rats alongwith biochemical and ultrastructural investigations. The toxicologic and cardiotoxic effects of single and divided high dosages of adriamycin given by intravenous injection were evaluated in 34 days old Newzealand Black Rats. Adriamycin at dosages of 20 mg per kg, 10 mg per kg X 2, 13 mg per kg, and 10 mg per kg produced fatality and

rapid weight loss followed by gradual weight gain in groups of rats that survived the 28 days observation period. Adriamycin at dosages of 5 mg per kg X 2 and 5 mg per kg produced no mortality but rats had attenuated weight gain compared to saline injected controls. The earliest myocardial fine structural alterations included swelling and degeneration of mitochondria and dilatation of sarcoplasmic reticulum at all dosages of adriamycin. More advanced myocardial lesions included separation of myofibrils and the fascial adherens of intercalated discs in rats 4 days after administration of 10 mg per kg of adriamycin.

The most severe lesions were observed in rats 8 days after receiving 10 mg per kg X 2 of adriamycin and included focal myocyte degeneration with increased electron density and contraction of the sarcomeres, nuclear pyknosis, and mitochondrial degeneration. Toxic dosages of adriamycin produced an acute, reversible hypocalcemia in rats. Significant increases of serum creatine phosphokinase and lactic dehydrogenase preceded the onset of highest mortality following adriamycin administration. Ventricular tissue calcium concentration was significantly increased in groups of rats receiving toxic dosages of adriamycin. This investigation demonstrates that the rat is sensitive to the cardiotoxic effects of single or divided high dosages of adriamycin.

The study shows that the rat is sensitive to the cardiotoxic effects of single or divided high dosages of adriamycin given by intravenous injection. Fatal dosages of adriamycin either produced acute weight loss or attenuation of weight gain, acute reversible hypocalcemia, and fine structural alterations compatible with severe myocardial damage. Significant increase of myocardial tissue calcium and serum enzymes (CPK, LDH) in association with myocardial damage were found to precede the onset of highest mortality rate. The decrease in mortality and concomitant increase in body weight gains with decreasing dosages of adriamycin suggest that the pathogenic mechanisms related to morbidity and mortality are both dose and time dependent. These effects also are dependent upon scheduling of adriamycin treatment, since dividing the total dosage into two boluses given at 48 hours intervals consistently results in prolongation of the interval from dosing until death. The debilitation and death associated with high dose adriamycin treatment suggests a multifactorial process including anorexia and wasting associated with gastrointestinal toxicity and bone marrow suppression. The rat therefore also appears to be sensitive, short term predictor for cardiotoxicity of these drugs in man.

Bristow et al (1978) collected data on all patients hospitalized at Stanford University Hospital between June, 30, 1976 and July 1, 1977 who had recently received their first

or second course of an anthracycline antibiotic (doxorubicin or daunorubicin). From these data eight patients were selected in whom (1) other precipitating causes of myocardial dysfunction could not be identified and (2) a similar type of cardiotoxic manifestations has been reported in animal models. Clinical observations revealed that of the eight patients identified as having early anthracycline cardiotoxicity, the first four patients presented with pericarditis and three patients had objective evidence of severe cardiac dysfunction. These patients were all relatively young and had no known history of cardiac disease, with the exception of one patient in whom pericarditis developed 24 hours after a single dose (60 mg/m^2) of daunorubicin. In contrast four patients were elderly and three of them had evidence of previous myocardial dysfunction. All these four patients presented with left sided heart failure. In one patient electrocardiographic evidence of anterior wall myocardial infarction developed.

Histopathology was available in two of these patients which showed evidence of acute inflammatory process involving the pericardium and epimyocardium. Four patients had clinical and chest X-ray evidence of heart failure developing shortly (one to 10 days) after the administration of anthracycline. The most obvious finding was that in the

absence of risk factors. Anthracycline associated heart failure is extremely uncommon at doses less than 550 mg/m^2 and myocyte damage is usually minimal at doses less than 300 mg/m^2. The mean total dose given to their patients was 137 mg/m^2.

Starkebaum (1975) and Harrison (1976) have independently described a 41 year old woman in whom pericarditis, myocarditis, fatal pump failure and cardiac arrest developed nine days after treatment with daunorubicin given as three daily 70 mg/m^2 doses.

Ippolito (1976) reported the development of cardiogenic shock refractory to therapy in 56 year old man one week after he received 80 mg total dose of daunorubicin, the same investigators reported a case of apparent myocardial infarction 48 hours after the patient received 50 mg dose of daunorubicin. Ainger et al (1971) reported a case of fatal pump failure in a 12 year old child 12 days after completion of a 250 mg/m^2 course of daunorubicin therapy given over five days. Shortly before death, the patients electrocardiogram showed an anterior injury pattern.

Rinehart et al (1974), Greco et al (1976) and Singer et al (1978) have described transient deterioration in left ventricular function following doses of d xorubicin.

Billingham, et al (1978) obtained percutaneous transvenous endomycardial biopsy from 60 patients aged (19-78 years) receiving adriamycin. The tissue obtained by endomycardial biopsy was usually not more than 3 mm in maximum dimensions. The rapidly fixed tissue provides excellent artifact free myocardium for conventional microscopy as well as optimal tissue for ultrastructural examination. In the earliest lesions, single cells with advanced degenerative changes could be seen isolated against a back ground of morphologically intact myocardium. The subendocardium and trabeculae represented a particularly prominent site of involvement in the early stages. Inflammatory infiltrate was not seen even in very severe lesions. Light microscopic changes included an increase in interstitial fibrosis from normal; however this change is dose dependent and not always present. There are two main types of myocyte injury which occur. The first is myofibrillar loss which is partial to begin with but which may become total. Myofibrillar lysis can also be recognized by light microscopy as smaller, shrunken cells with homogenous pale cytoplasm. In the second type of myocyte damage, the cell undergoes vacuolar degeneration. The earliest manifestation is distension of the sarcoplasmic reticulum which eventually swells and coalesce to form large membrane bound clear spaces in the cytoplasm. This change can also occur with preservation of the mitochondria and nucleus.

These lesions eventually progress until the death of the myocyte at which time the mitochondria do degenerate by swelling and cristolysis, myelin figures appear, and the nuclei become pyknotic and disintegrate. In their study, the endothelial cells of the cappilaries and small arterioles, as well as the nerve endings, were found to be unaffected when compared with normal control biopsies, even if they were from patients who had received maximal dose of anthracycline. In addition their data showed neither depletion nor increase in intramyocyte glycogen in severe, anthra cycline induced cardiotoxicity.

Bertazoll et al (1979) worked for quantitative experimental evaluation of adriamycin cardiotoxicity in the mouse. Histologic patterns of control and treated mice, showed different degrees of degenerative lesions. Both severity and extension of the lesions were dose related. At the lowest dose 2 mg/kg given twice a week for 5 week, the microscopic lesions were represented by characteristic microvacuolization of fibres, the injury involved few myocytes. When a total I/V dose of 40 mg/kg of adriamycin, as a single I/V dose of 4 mg/kg was given twice a week for 5 weeks, vacuolization, myolysis, myofiber atrophy and focal interstitial fibrosis were evident. These lesions were generally accompanied by a more extensive involvement of the myocardium.

In addition, swelling of single endocardial cells and thickening of the endocardium were observed in the atria.

Taylor et al (1982) tried to determine the very early changes in subcellular structure caused by adriamycin in an isolated, perfused, working heart. Eighteen rabbit hearts were perfused with oxygenated Krebs Ringer Bicarbonate buffer at 39°C containing either 1, 2, 4 or 8 mg per litre of adriamycin for periods of 30 to 150 minutes, in all twenty eight hearts were studied, there were 10 control hearts and 18 hearts exposed to adriamycin. In all hearts contractile function persisted throughout the experimental period, but anthracycline exposed hearts showed greater variability in function with time than did control hearts. Light microscopic findings were as follows (1) Separation of myofibres consistent with interstitial edema (2) Focal contraction band necrosis was present (3) Cytoplasmic and particularly perinuclear vacuolization with 17 of 18 hearts showing scattered vacuolization of myocardial cells and nine of 18 (50 percent) showing marked alterations with some vacuolization evident in virtually every low power (X10) field studied (4) No nuclear alterations were seen by light microscopy. Electron microscopic findings of hearts exposed to adriamycin consisted of disruption of sarcomeres with myocytolysis, cytoplasmic vacuolization,

and swelling of mitochondria with occasional presence of flocculent densities. A distinctive, central clumping of the nuclear material with clearing of chromatin along the nuclear membrane was evident in the nuclei of all cells from adriamycin exposed hearts. Other findings noted in small numbers of treated hearts were myelin bodies in one heart and prominent lipid granules in four hearts. In no instance was the central nuclear clumping with clearing of chromatin from the nuclear membrane occuring in the adriamycin hearts seen in the control hearts.

Van Vleet et al (1986) reported that gross lesions of carditoxicity in pigs, rabbits and dogs were (1) hydropericardium (2) hydrothorax (3) Ascitis. In occasional pigs, fibrinous pericarditis was present. The myocardium was pale, and hearts were dilated when compared with control hearts.

The three major lesions observed in myocytes were (1) Sarcoplasmic vacuolization (2) Myocytolysis (3) Hyaline necrosis.

Van Vleet et al (1980) studied cardiac disease induced by chronic adriamycin administration in dogs and an evaluation of Vitamin E and selenium as cardioprotectants. Chronic adriamycin intoxication was produced in three groups. of beagle dogs by weekly intravenous injections (1 mg/kg body

weight) for 20 weeks (cumulative dose 400 mg/sq m). Group A (6 dogs) received adriamycin only; Group B (6 dogs) were given adriamycin and weekly doses of Vitamin E (17 mg/kg body weight) as alpha tochopherol acetate, and Group C (6 dogs) received adriamycin and weekly doses of Vitamin E as did Group B and selenium (0.06 mg/kg body weight as selenite). Each of the 18 dogs developed adriamycin induced cardiomyopathy, and death occured in 11 dogs during weeks 17-20. Mortality was lowest in Group B (2 of 6), but no differences between groups were seen either in survival time of the dogs that died or in severity of cardiomyopathy. Cardiomyopathy was more severe in dogs that died than in survivors. Congestive heart failure with transudation was present in 4 of 11 dogs that died. Cardiac histopathology was characterized by vacuolar degeneration of myocytes. Myocardial damage was most severe in the left ventricle and the ventricular septum, intermediate in the right ventricle and the left atrium, and least in the right atrium. Ultrastructural study showed that an early alteration in damaged myocytes was distension of sarcoplasmic reticulum to form sarcoplasmic vacuoles. Occasional damaged fibres had myofibrillar lysis and focal proliferation of sarcoplasmic reticulum. The lack of cardioprotection from Vitamin E and selenium supplementation fails to support the proposed role of lipoperoxidative damage in the development of chronic adriamycin induced cardiomyopathy.

Bristow et al (1981) studied anthracycline associated cardiac and renal damage in rabbits. The authors tested the hypothesis that anthracycline induced cardiac and renal damage is mediated by vaso active substances. A 1 minute exposure to 5 ug per ml of doxorubicin produced cardiac histamine release in isolated rabbit hearts. Under conditions in which histamine uptake and metabolism were impaired, the administration of doxorubicin, 2 mg/kg, over 1 minute was associated with elevations in arterial histamine and catecholamines. The chronic weekly administration of doxorubicin produced severe cardiac and renal damage. The administration of combined histamine and adrenergic blockade with diphenhydramine, cimetidine, phentolamine and propranolol pre and immediately post doxorubicin resulted in near total protection against doxorubicin mediated cardiac damage and prevented the majority of the renal lesions. The observations that (i) doxorubicin in a dose that produces a cardiomyopathy and causes cardiac histamine release and elevations in systemic levels of histamine and catecholamines and (2) pretreatment with agents that block histamine and catecholamine receptors prevents doxorubicin related chronic cardiac damage strongly suggests that at least part of anthracycline associated cardiac toxicity is mediated by vasoactive substances. Therefore, the evidence supports the original hypothesis that the acute and chronic cardiovascular effects of anthracyclines may be related to a common mechanism, the release and increased

Jackson et al (1984) carried out experiments to evaluate free radical effects and catecholamine alterations in adriamycin cardiotoxicity. In acute studies, adriamycin treated rabbits exhibited significantly increased levels (upto 50%) of total and reduced glutathione, unchanged levels of oxidized glutathione, and a slight decrease in the percentage of oxidized glutathione. In the chronic study (dose 1.1 mg/kg twice weekly per 10 weeks) levels of total and reduced glutathione were increased significantly by 23-36% after 9-12 and 16-20 injections without change in the percentage of oxidized glutathione peroxidase activity was not reduced significantly in any group of acute or chronic adriamycin treated animals. Tests for lipid per oxidation (malondialdehyde and ethane production) were negative in acute studies. Myocardial catecholamine levels were unchanged in acute and chronic adriamycin treated animals. Thus (1) the cardiac glutathione glutathione peroxidase system in activated with adriamycin treatment at the onset of cellular damage, and (2) cellular damage progresses without further alteration of this system, loss of glutathione per oxidase activity, or reduction in myocardial catecholamines in rabbit models of adriamycin cardiotoxicity. These findings suggest that free radical generation in the heart may contribute to adriamycin cardiotoxicity, but that other factors probably play a more important role in pathogenesis of the myocardial damage.

MATERIAL AND METHOD

MATERIAL AND METHOD

EXPERIMENTAL ANIMALS

The experiments were performed on 42 healthy Swiss albino rats of both sexes weighing 100 to 200 gms obtained from Animal House at Maharani Laxmi Bai Medical College, Jhansi. The animals were maintained on standard balanced diet.

The animals were devided into 3 groups of 12 animals each; 6 untreated animals served as healthy control.

ADMINISTRATION OF DRUG

Doxorubicin hydrochloride (trade name Doxorubicin Meiji) manufactured by Dong - A - Pharma. Co. Ltd., Seoul, KOREA marketed in India by Biochem Pharmaceutical Industries, Bombay, was used; 10 mg vial of Doxorubicin hydrochloride was reconstituted with 5 ml sterile normal saline. The reconstituted solution is stable for 24 hours at room temperature and 48 hours under refrigeration (2° - $8^{\circ}C$). Any unused solution was discarded.

Thirty six rats divided into three groups A, B and C of 12 animals each received the drug intraperitoneally (i.p.) as per following schedule :-

Animals of Group 'A' were given a single intraperitoneal injection of 10 mg/kg doxorubicin, whereas those of Group 'B' received i.p.i. of doxorubicin in doses of 5 mg/kg on two consecutive days and the animals of Group 'C' were administered with doxorubicin in doses of 1 mg/kg on ten consecutive days.

Sacrifice of Animals :

After the last injection the animals were sacrificed in batches on day 1, 3, 7 and 14 under ether anesthesia. At autopsy the heart along with covering pericardium was removed carefully and washed thoroughly in cold isotonic normal saline. Its gross features were recorded. The hearts were preserved in 10% formal saline for detailed histopathological examination.

HISTOLOGY OF HEART

From each heart four serial transverse parallel blocks including one at the apex, another from ventricles, the third from atrioventricular junction and the last from base of the heart, so as to include large blood vessels, were obtained. From each block 3-5 serial paraffin sections 3-4 um thick were obtained and stained with Harris's Haematoxylin and Eosin; V.G., Reticulin and PAS stains were also used whenever needed.

The sections were examined in detail using a light microscope and the findings of the histological examination in terms of cytoplasmic vacuolization, myofibre necrosis, inflammatory and mesenchymal reactions and interstitial haemorrhage were recorded and arbitrarilly graded using half point scale. The distribution and nature of these lesions was also recorded. The severity of the lesions was graded as 'Mild' (+), 'Moderate' (++), or 'Marked'(+++) as described below :

The individual sections were graded separately and the average findings were expressed in results. Associated findings e.g., pericarditis, valvulitis, lymphatic involvement and vascular changes were also noted. The observations were recorded using half point scale as detailed below. The changes were graded on a scale of 0 to 1.5 corresponding to absent, mild, moderate and marked.

CYTOPLASMIC VACUOLIZATION :

0	:	No vacuolization seen
0.5	:	Isolated minimal foci of characterististically vacuolated myocytes.
1	:	Larger and more numerous foci of vacuolated myocytes.
1.5	:	Generalized vacuolization of myocytes covering more than 75% of the high power field.

MYOFIBRE NECROSIS :

Mild (+) : Scattered small foci consisting of 1 to 2 necrosed myofibres.

Moderate (++) : Scattered foci consisting of 3 to 5 necrosed myofibres.

Marked (+++) : Confluent foci of myofibre necrosis or when scattered, larger foci consisting of more than 5 necrosed myofibres.

INFLAMMATION

Mild (+) : Focal collection of less than 10 inflammatory cells.

Moderate (++) : Focal collection of 10-20 inflammatory cells.

Marked (+++) : Diffuse infiltration with inflammatory cells in a wide area or focal collection of more than 20 cells.

HAEMORRHAGE :

The interstitial haemorrhages, which were few or multiple, depending upon their size were arbitrarily graded as small or large.

INJURY SCORE

In order to have some quantitative assessment of myocardial injury in different rats, the attempt was made for numerical scoring of the injury. For this purpose the points awarded to a lesion on half point scale were added and their sum total represented the injury score for a particular section. Average score of all the sections represented the injury score of that particular heart, and the mean injury score of a particular batch and/or a group of animals, thus could be calculated and used in further assessment and comparison of intensity and severity of myocardial lesions in various groups of these animals.

OBSERVATIONS AND RESULTS

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GROSS FINDING :

The heart of the animals sacrificed at various periods after i.p.i. of dexorubicin in varying doses did not reveal any gross pathological changes. Attempts to ascertain the gross pathological changes in the valves were unsuccessful due to the small size of the organ.

MICROSCOPIC FINDINGS :

Three to five serial sections obtained at different levels of the heart stained with haematoxylin and eosin were thoroughly examined for the presence of the cytoplasmic vacuolization, myofibre necrosis, inflammatory reaction and the interstitial haemorrhage. The distribution and the nature of the lesions was recorded and depending upon the severity and extent of myocardial damage, the histological findings of cardiac lesions in terms of cytoplasmic vacuolization were graded using half point scale as 0.5, 1, 1.5 and those of myofibre necrosis and inflammatory reaction were arbitrarily graded as mild (+), moderate (++) and marked (+++). The individual sections were graded separately and the average findings have been reported in results. Associated histological findings of cardiac lesions such as pericarditis, valvulitis, papillary muscle involvement and contracture band necrosis were also recorded.

GROUP 'A' :

Histological findings in heart of animals of group 'A' who received single i.p.i. of 10 mg/kg of Doxorubicin have been shown in detail in Table I.

Animals of this group sacrificed on day one after a single i.p.i. of 10 mg/kg doxorubicin showed morphologic cardiac lesions in all of them. The heart of all the animals had foci of vacuolated myocytes (grading 0.5) along with mild (+) inflammatory reaction. One of these animals who had multiple interstitial haemorrhages also showed pericarditis as well as valvulitis, while other two animals had only small interstitial haemorrhages.

The severity of the lesions was found to have increased in animals sacrificed on day three and seven after the administration of the drug, as almost all these animals had small foci of myofibre necrosis associated with mild or moderate inflammatory reaction. Small interstitial haemorrhages were present in almost all the animals and all of them had pericarditis as well as valvulitis.

Papillary muscle involvement was also observed in three animals. The severity of the lesions showed regression in animals sacrificed fourteen days after the administration of the drug, as these animals had mild myofibre necrosis alongwith mild inflammatory reaction. They

also had small interstitial myocardial haemorrhages and the pericarditis and valvulitis was seen in only half of them.

Four animals of this group, also showed contracture band necrosis in the left ventricular muscle. In all the animals there were dialated and congested blood vessels, few of them showing leakage and extravasation of blood. The vascular changes were more marked in the subepicardial zone. Out of eleven animals three showed papillary muscle involvement. Most of the animals (9/11) showed multiple small areas of haemorrhages while two rats sacrificed on day 1 and 7 respectively showed large areas of interstitial haemorrhage. Pericarditis and valvulitis with or without involvement of the valvering was encountered in seven out of 11 animals. The intensity of myocardial injury gradually increased as the animals sacrificed at day one, showed only cytoplasmic vacoulization where as those sacrificed at day 3 and 7 had myofibre necrosis; although the severity of cardiac injury regressed at day 14 after the administration of the drug. However, occasional foci of myofibre necrosis were still seen in these animals.

The injury score in rats sacrificed on day one after the administration of the drug was 1.5 in all the rats thus average injury score of the batch was 1.5. The

average batch score of animals sacrificed on day 3rd and 7th was 1.7 and 2.3 respectively thus indicating a gradual increase in the intensity of injury. The average injury score for animals sacrificed on day 14 after the drug administration fell to 1.75, thus indicating the regression in the intensity of the injury produced by the drug. The average score derived for all the animals of the group was 1.81.

GROUP 'B' :

Histological findings in heart of animals who received doxorubicin in doses of 5 mg/kg i.p.i. for two consecutive days have been shown in detail in Table II.

Animals of this group sacrificed on day one after two consecutive i.p.i. of 5 mg/kg doxorubicin showed morphologic cardiac lesions in all of them. The heart of two animals had foci of vacuolated myocytes (grading 0.5) among with minimal to mild degree of inflammatory response; one of these animals also exhibited mild myofibre necrosis. Small interstitial haemorrhages were present in almost all the animals while pericarditis and valvulitis was observed in two of them. One animal of this sub group also showed papillary muscle involvement. The severity of the lesions was found to be almost similar in animals sacrificed on day three. The severity of the lesions was found to have increased in animals sacrificed

on day seven after the administration of the drug. Two third of these animals had small foci of myofibre necrosis associated with mild inflammatory reaction. Small interstitial haemorrhages were present in all the animals and all of them had pericarditis valvulitis and papillary muscle involvement. The animals sacrificed at later interval showed regression in the severity of the lesions, as the heart of these animals had only foci of vacuolated myocytes (grading 0.5) along with mild inflammatory reaction; none had myofibres necrosis. One of the animals who had large interstitial haemorrhage also showed pericarditis as well as valvulitis while other two animals had only small interstitial haemorrhages. One animal of this group also showed papillary muscle involvement.

In all the animals there were some dialated and congested blood vessels, few of them showed leakage with extravasation of blood, these vascular changes, as in the previous group, were more pronounced in the subepicardial zone. Out of twelve animals in this group, five showed papillary muscle involvement. The extent of myofibre necrosis was reduced in animals sacrificed later but the extent of inflammatory reaction showed little change. Most of the animals (9/12) showed multiple small areas of interstitial haemorrhage while one rat sacrificed at day fourteen after

the administration of the drug showed large areas of interstitial haemorrhage. Pericarditis was seen in ~~eleven~~ ten out of twelve animals and valvulitis with or without involvement of valve ring was observed in ten out of twelve animals. Cellular oedema and wide separation of sucle fibres was also seen occasionaly.

The average injury score derived for rats sacrificed on day 1st and 3rd after the drug administration was found to be 1.5 on both occasions. There was a rise in the average injury score (1.8) for the rats sacrificed on day 7th while it regressed to 1.5 in the animals sacrificed on 14th day after the drug administration.

GROUP 'C' :

Histological findings in heart of animals of group 'C' who received doxorubicin in doses of 1 mg/kg i.p.i. for ten consecutive days have been shown in detail in Table - III.

Animals of this group sacrificed on day one after ten sonsecutive i.p.i. of 1 mg/kg doxorubicin showed morphologic cardiac lesions in all of them. The heart of all the animals had mild myofibre necrosis along with mild or moderate inflammatory reaction. Two of these animals who had

large foci of interstitial haemorrhage also showed pericarditis as well as valvulitis having papillary muscle involvement in one of them while one animal of this sub group had only small interstitial haemorrhages with no pericardial involvement.

Animals sacrificed at later intervals showed almost similar pattern of histological changes and the severity of the lesions was also found to be more or less the same.

All the animals in this group showed mild degree of myofibre necrosis. Of the twelve animals four showed moderate degree of inflammatory reaction while the eight showed mild inflammation. Pericarditis was seen in nine animals and valvulitis was obserbed in most of the animals (11/12) with or without involvement of the valve ring. One animal sacrificed at day one showed pericarditis and valvulitis. Six animals showed papillary muscle involvement. Large foci of haemorrhage were seen in four rats while small multiple foci were observed in five of them. Seven out of twelve animals showed dilated blood vessels mainly in the subepicardial zone with or without rupture.

As evident from the injury score (Table- III) the intensity of the lesions produced in animals sacrificed at different intervals after i.p. administration of doxorubicin in doses of 1 mg/kg for 10 consecutive days was almost same.

Animals in all the three groups receiving i.p.i. of doxorubicin in different doses showed morphologic cardiac lesions in all of them. Small interstitial haemorrhages were encountered in most of the hearts with occasional large foci of interstitial haemorrhages. Four out of twelve animals in group 'C' showed large foci of interstitial haemorrhage thus accounting for maximum frequency. Foci of vacuolated myocytes (grading 0.5) were seen in the heart of eight out of twelve animals in group 'B' and four out of 11 animals in group 'A' while none of the animals of group 'C' showed cytoplasmic vacuolization. Mild myofibre necrosis and mild to moderate inflammatory response was seen in most of the animals. All the animals (12/12) of group 'C' showed mild myofibre necrosis with mild inflammatory response in (8/12) animals while moderate response in (4/12) animals as compared to mild myofibre necrosis (9/12) alongwith mild inflammatory response in (10/12) animals of group 'B', while in group 'A' only (6/12) showed mild myofibre necrosis along with mild inflammatory reaction in (9/12) animals. Pericarditis and valvulitis with or with out involvement of the ring was a frequent feature in all groups.

The injury score revealed that intensity of the myocardial injury in group 'A' and 'B' was almost similar while group 'C' animals had most pronounced cardiac injury. Papillary muscle involvement was seen in quite a number of cases; group 'C' animals showed maximal involvement with 6/12 animals showing this lesions, while 5/12 animals of group 'B' and 3/12 animals of group 'A' showing papillary muscle lesion.

Contracture band necrosis was a frequent feature in the animals of group 'A', where (4/11) animals exhibited the contracture band necrosis. Only single animal in group 'B' and none in group 'C' showed contracture band necrosis.

Table - I : Shows histological findings in heart of rats of Group 'A' treated with Doxorubicin in doses of 10 mg/kg single i.p.i.

Day of Sacrifice	No. of Rats	Interstitial Haemorrhage	Cytoplasmic Vacuolization	Myofibre Necrosis	Inflammation	Contracture Band Necrosis	Papillary Muscle Involvement	Pericarditis	Valvulitis	Injury Score	
	1	L	0.5	-	+	-	-	P	P	1.5	
1st	2	S	0.5	-	+	-	-	A	A	1.5	(1.5)
	3	S	0.5	-	+	-	-	A	A	1.5	
	1	S	-	+	+	-	-	A	A	2.0	
3rd	2	S	0.5	-	±	-	-	P	P	1.0	(1.7)
	3	S	-	+	+	-	+	P	P	2.0	
	1	S	-	+	++	-	+	P	P	3.0	
7th	2	L	-	+	+	+	+	P	P	2.0	(2.3)
	3	S	-	+	+	+	-	P	P	2.0	
	1	S	-	+	+	+	-	P	P	2.0	
14th	2	S	-	±	+	+	-	A	A	1.5	(1.75
	3	---------- Died during experiments ----------									

S = Small, L = Large, P = Present, A = Absent

( ) figures within paranthesis indicate average injury score of the batch.

Table - II : Shows histological findings in the heart of rats of Group 'B' treated with Doxorubicin in doses of 5 mg/kg i.p.i. on 2 consecutive days.

Day of Sacrifice	No.of Rats	Interstitial Haemorrhage.	Cytoplasmic Vacuolization	Myofibre Necrosis	Inflammation	Contracture Band Necrosis	Papillary Muscle Involvement	Pericarditis	Valvulitis	Injury Score	
	1	S	0.5	-	+	-	-	A	A	1.5	
1st	2	S	-	+	+	-	+	P	P	2.0	(1.5
	3	S	0.5	-	±	-	-	P	P	1.0	
	1	S	-	+	+	-	-	P	P	2.0	
3rd	2	-	0.5	-	±	-	-	P	P	1.0	(1.5
	3	-	0.5	-	+	-	-	P	P	1.5	
	1	S	0.5	-	+	-	+	P	P	1.5	
7th	2	S	-	+	+	-	+	P	P	2.0	(1.8
	3	S	-	+	+	-	+	P	P	2.0	
	1	L	0.5	-	+	+	-	P	P	1.5	
14th	2	S	0.5	-	+	-	-	A	A	1.5	(1.5
	3	S	0.5	-	+	-	+	P	P	1.5	

S = Small, L = Large, P = Present, A = Absent

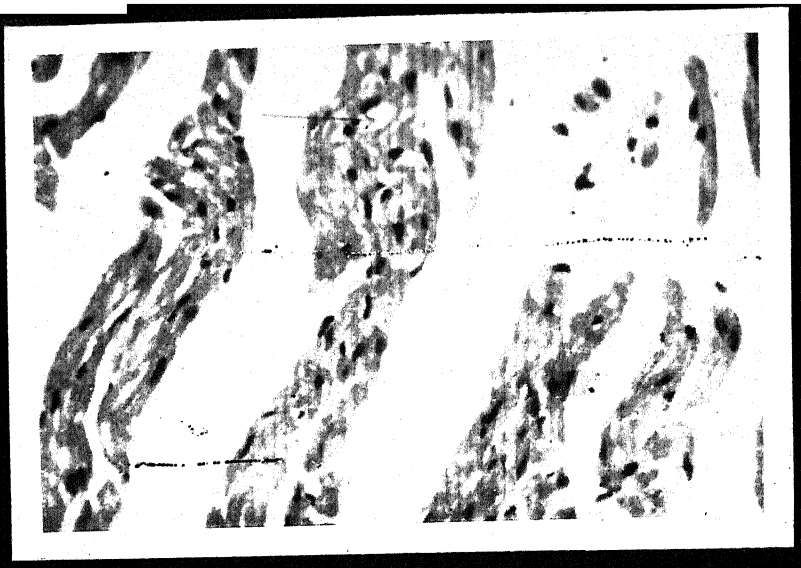
( ) figures within parenthesis indicate average injury score of the batch.

Table - III : Shows histological findings in heart of rats of Group 'C' treated with Doxorubicin in doses of 1 mg/kg i.p.i. on 10 consecutive days.

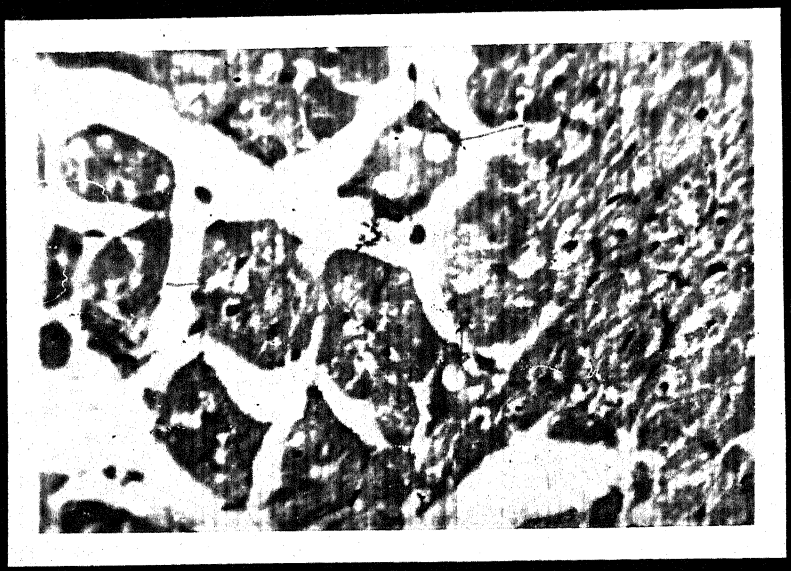
Day of Sacrifice	No.of Rats	Interstitial Haemorrhage	Cytoplasmic Vacuolization	Myofibre Necrosis	Inflammation	Contracture Band Necrosis	Papillary Muscle Involvement	Pericarditis	Valvulitis	Injury Score	
1st	1	L	-	+	+	-	++	P	P	2.0	(2.3)
	2	L	-	+	+	-	-	P	P	2.0	
	3	S	-	+	++	-	-	A	P	3.0	
3rd	1	S	-	+	+	-	++	A	A	2.0	(2.3)
	2	L	-	+	++	-	+	P	P	3.0	
	3	S	-	+	+	-	+	P	P	2.0	
7th	1	L	-	+	+	-	-	P	P	2.0	(2.3)
	2	S	-	+	++	-	-	A	P	3.0	
	3	S	-	+	+	-	++	P	P	2.0	
14th	1	-	-	+	+	-	+	P	P	2.0	(2.3)
	2	-	-	+	+	-	-	P	P	2.0	
	3	-	-	+	++	-	-	P	P	3.0	

S = Small, L = Large, P = Present, A = Absent

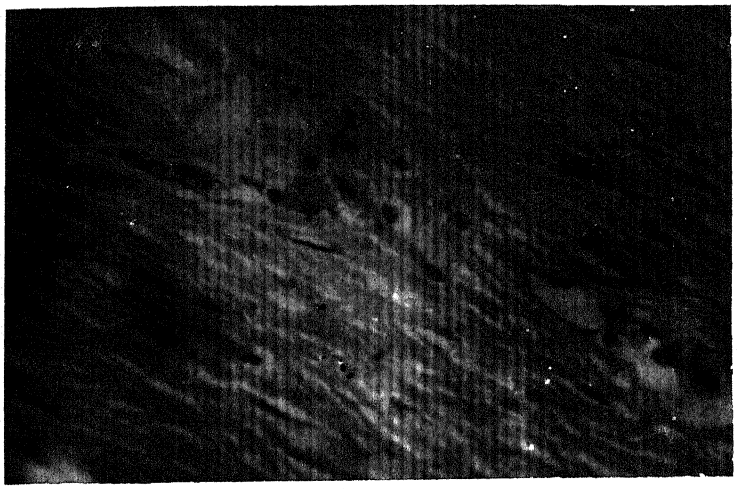
( ) figures within parenthesis indicate average injury score of the batch.



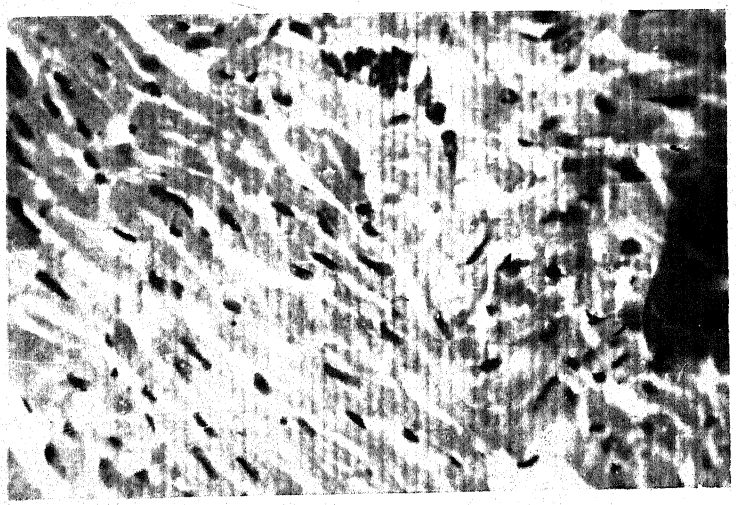
1 Microphotograph of rat heart 1 day after i.p.i. of 10 mg/kg Doxorubicin cytoplasmic vaculization. H & E X 280.



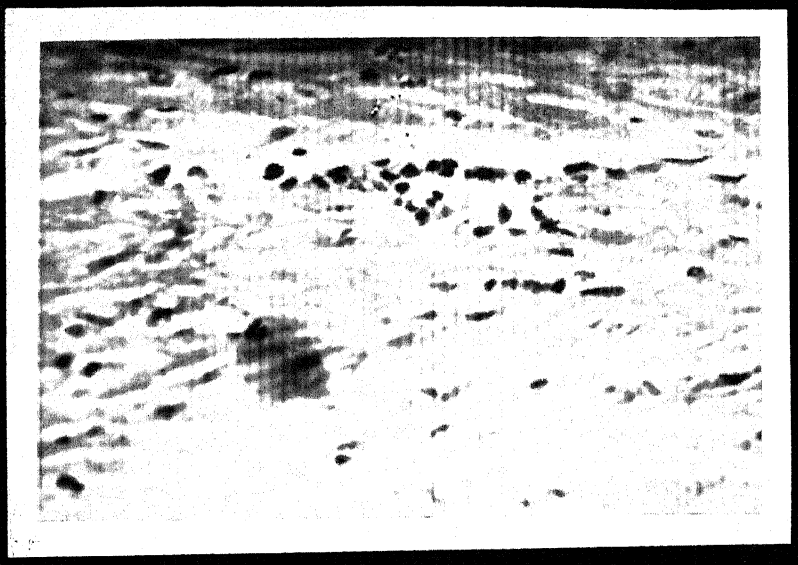
2 Microphotograph of rat heart 3 days after i.p.i. of 5 mg/kg Doxorubicin on 2 consecutive days showing intra cytoplasmic vacuolization. H & E X 280.



3 Microphotograph of rat heart 3 days after i.p.i. of 10 mg/kg Doxorubicin showing perinuclear vacuolization, inflammatory reaction H & E x 280.

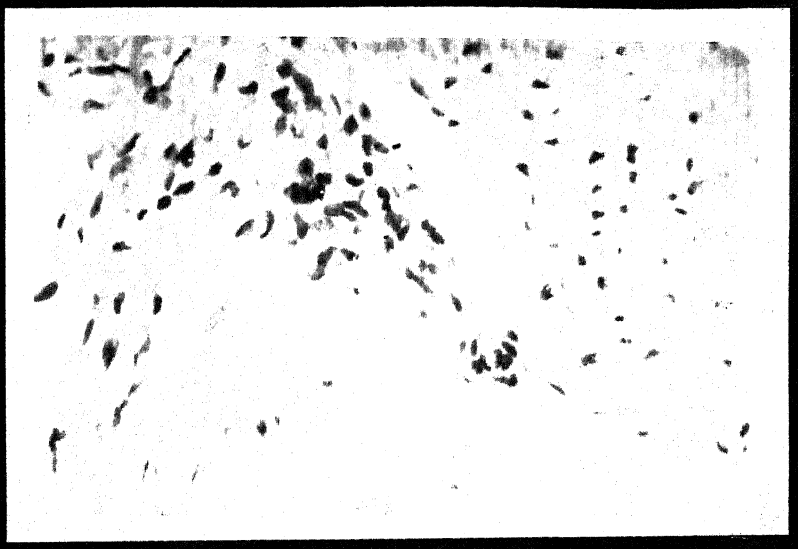


4 Microphotograph of rat heart 1 day after i.p.i. of 1 mg/kg Doxorubicin for 10 consecutive days showing mild myofibre necrosis and mild inflammation. H & E x 280.



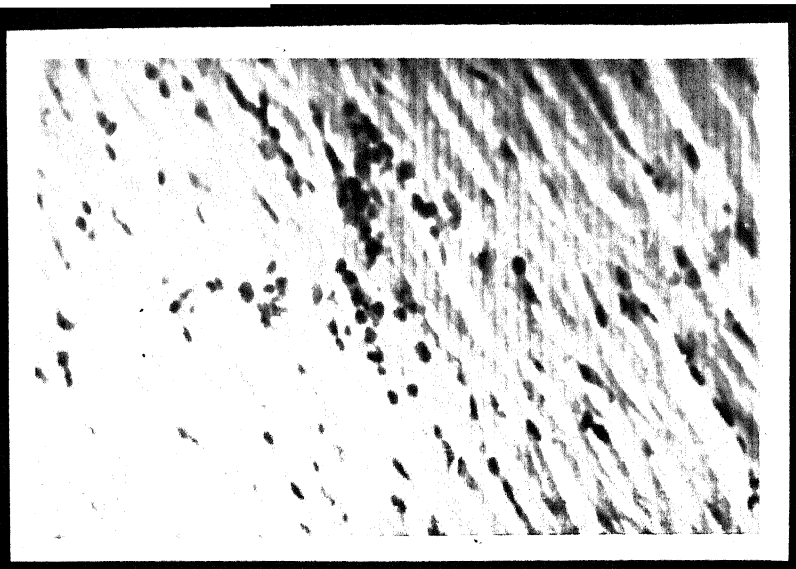
5. Microphotograph of rat heart 3 days after i.p.i. of 10 mg/kg Doxorubicin showing mild myofibre necrosis and mild inflammatory reaction.
H & E x 280.

6 Microphotograph of rat heart 3 days after i.p.i. of 1 mg/kg Doxorubicin on 10 consecutive days showing multiple foci of mild myofibre necrosis and mild inflammatory reaction.
H & E x 280.

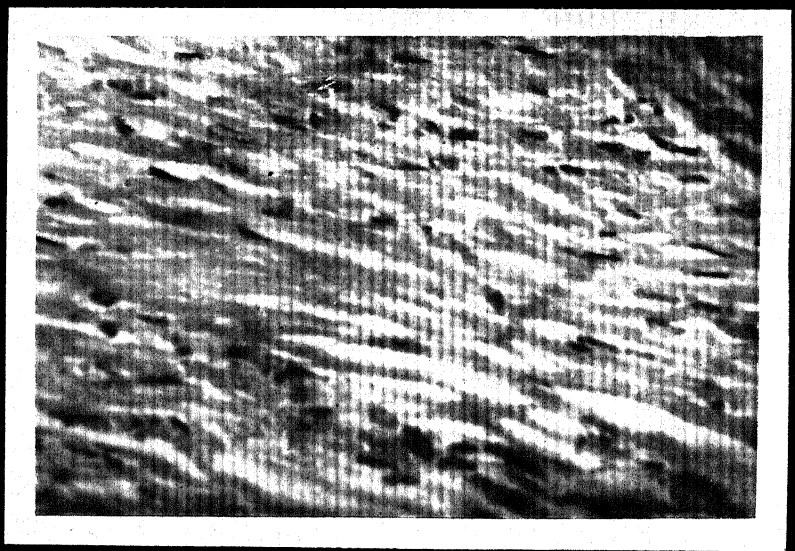


7 Microphotograph of rat heart 7 days after i.p.i. of 10 mg/kg Doxorubicin showing moderate inflammatory reaction. H & E x 280.

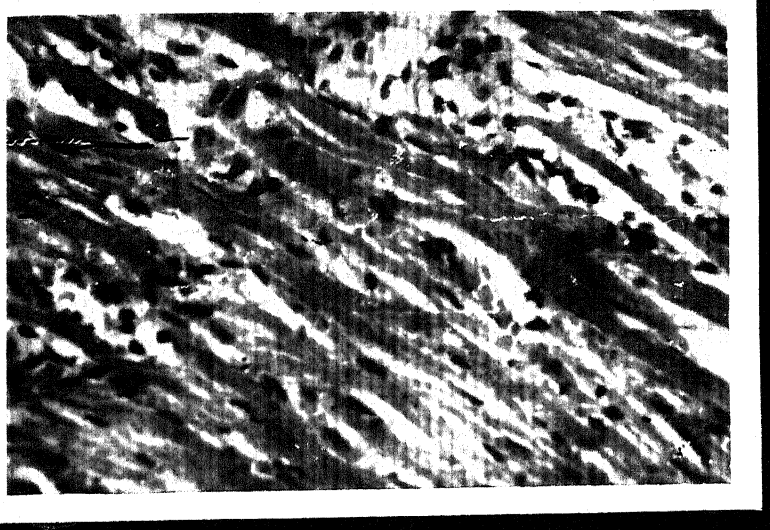
8 Microphotograph of rat heart 7 days after i.p.i. of 10 mg/kg Doxorubicin showing moderate inflammatory reaction. H & E x 280.



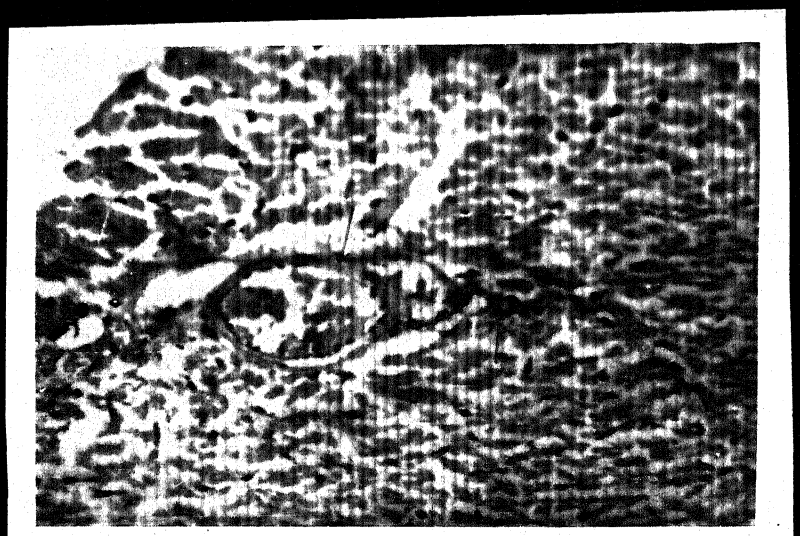
9. Microphotograph showing multiple confluent foci of myofibre necrosis of inflammatory reaction in a rat 7 days after 10 mg/kg i.p.i. of Doxorubicin. H & E x 280.



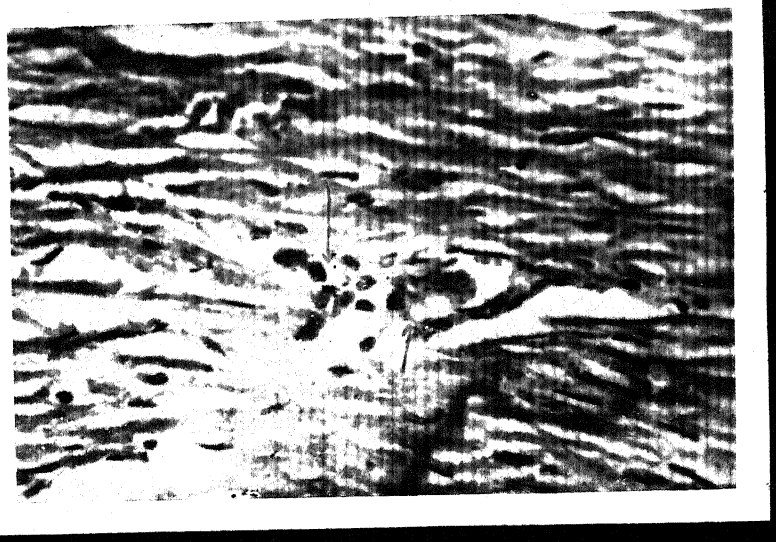
10. Microphotograph of rat heart showing mild myofibre necrosis and inflammatory reaction in a rat 1 day after 1 mg/kg i.p.i. of Doxorubicin for 10 consecutive days. H & E x 280.



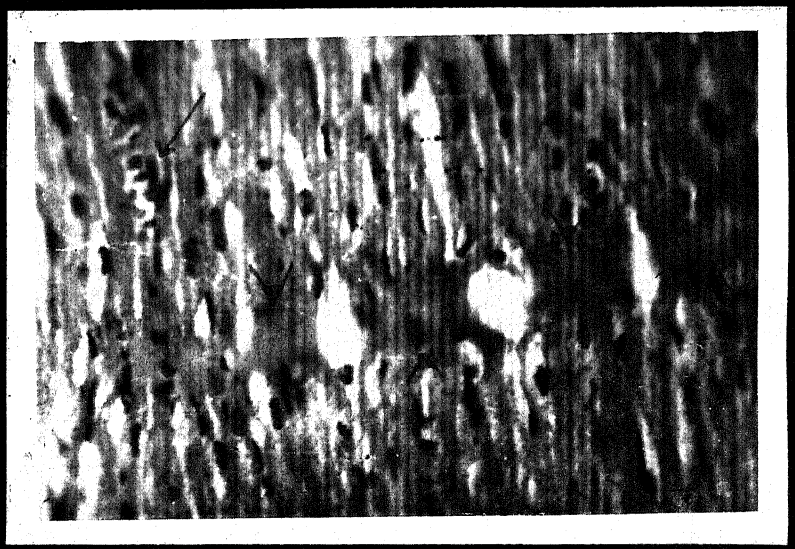
11 Microphotograph of rat heart 3 days after 1 mg/kg i.p.i. of Doxorubicin showing moderate myofibre necrosis and moderate inflammatory reaction. H&E x 280



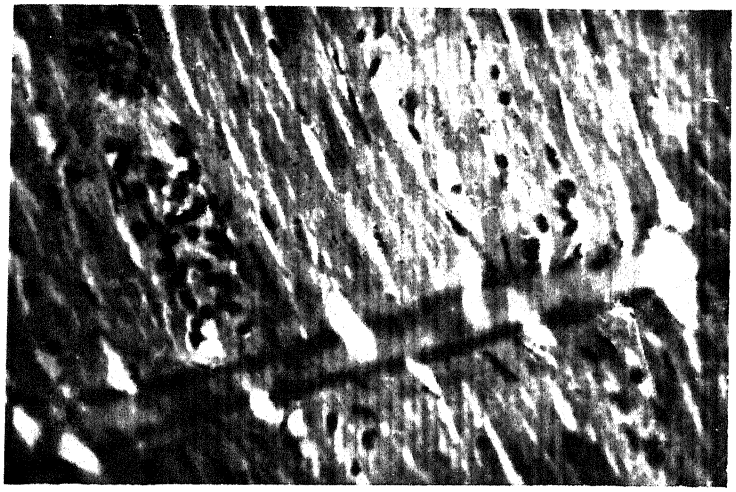
12 Microphotograph of rat heart 1 day after 1 mg/kg i.p.i.of Doxorubicin for 10 consecutive days showing vascular congestion & perivascular inflammatory reaction. H & E x 280.



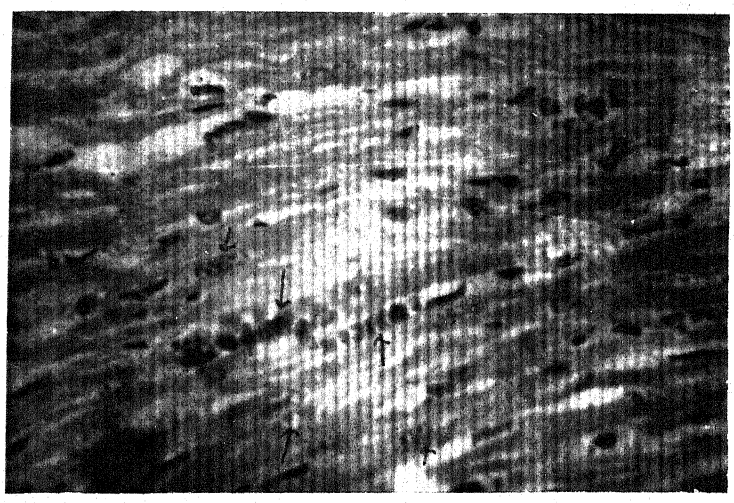
13 Microphotograph of rat heart 3 days after 1 mg/kg i.p.i.of Doxorubicin for 10 consecutive days showing breaking up of congested blood vessel & perivascular inflammatory reaction. H & E x 280.



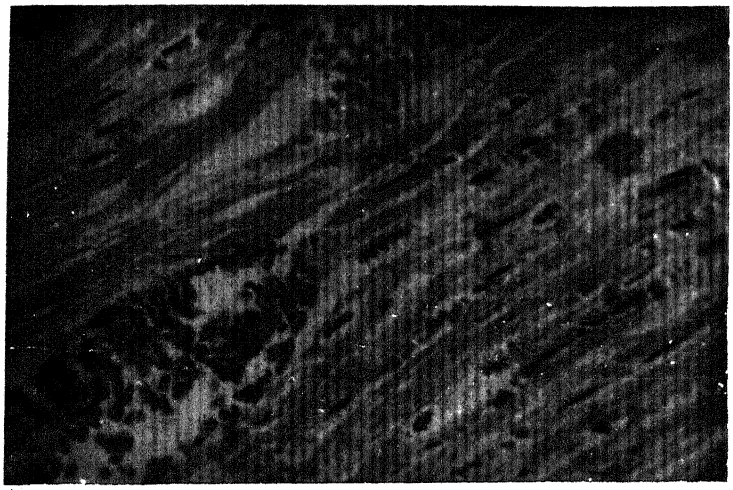
14 Microphotograph of rat heart 7 days after 10 mg/kg i.p.i.of Doxorubicin showing mild interstitial haemorrhage and contracture band necrosis. H & E x 280.



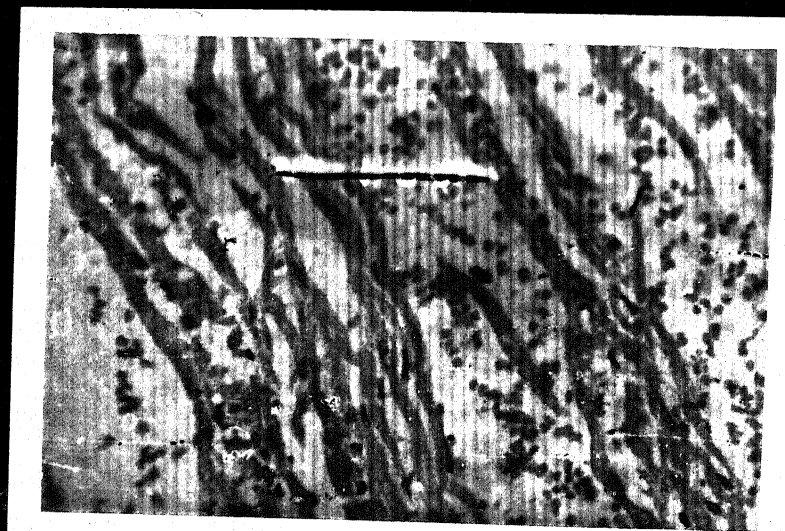
15 Microphotograph of rat heart 7 days after 10 mg/kg i.p.i.of Doxorubicin showing contraction band necrosis along with mild myofibre necrosis & inflammatory reaction H&E x 280.



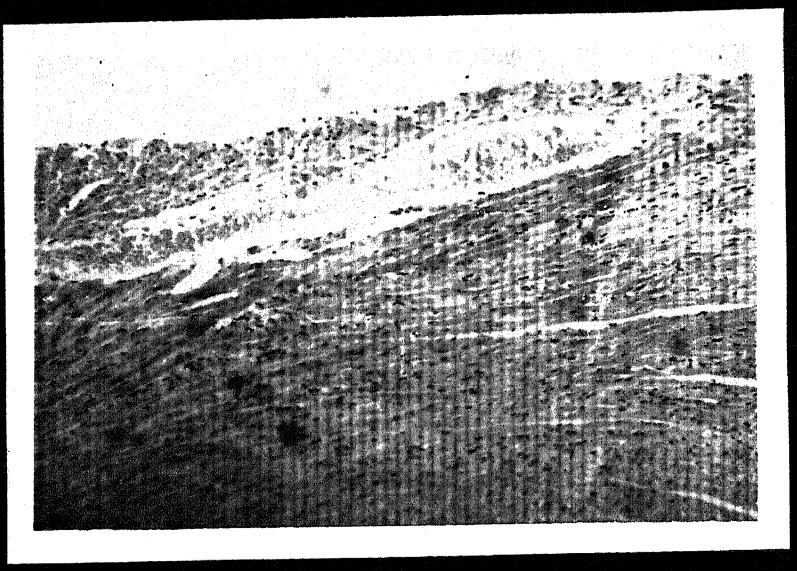
16 Microphotograph of rat heart 3 days after 10mg/kg i.p.i. of Doxorubicin showing multiple small interstitial haemorrhage. H & E x 280.



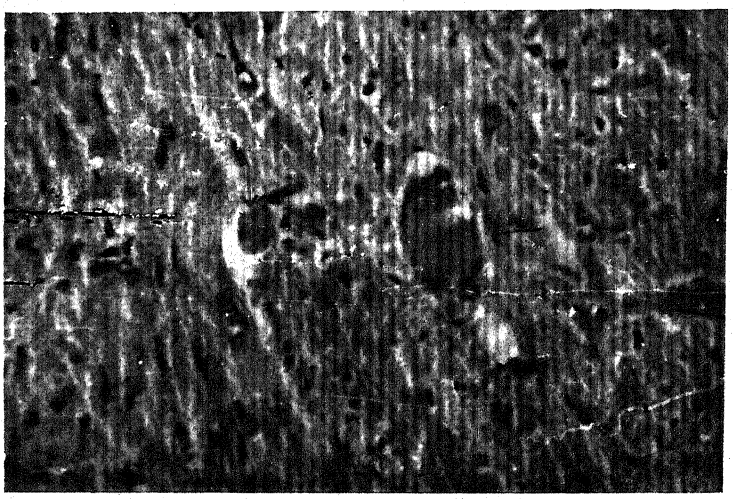
17 Microphotograph of rat heart 1 days after 1 mg/kg i.p.i.of Doxorubicin on 10 consecutive days showing large interstitial haemorrhages. H & E x 280.



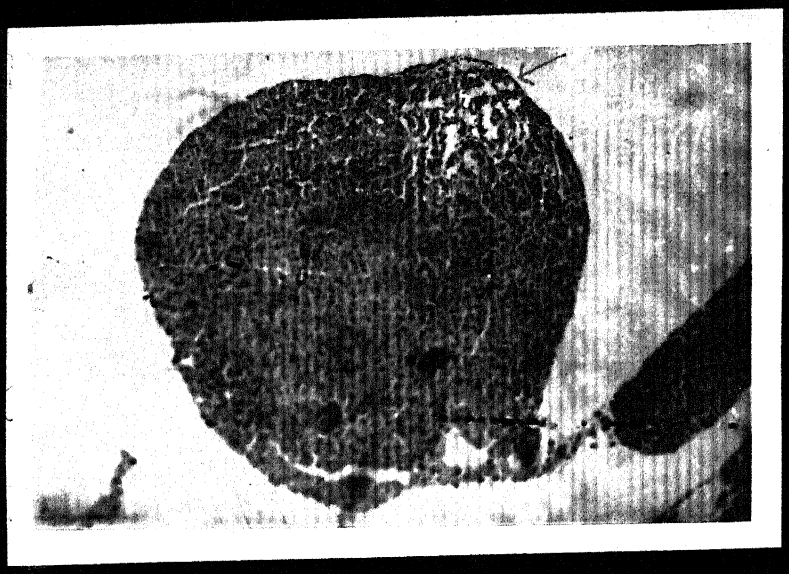
18 Microphotograph of rat heart 3 days after 1 mg/kg i.p.i.of Doxorubicin on 10 consecutive days showing multiple intramyocardial haemorrhage and intersititial edema H&E x 280.



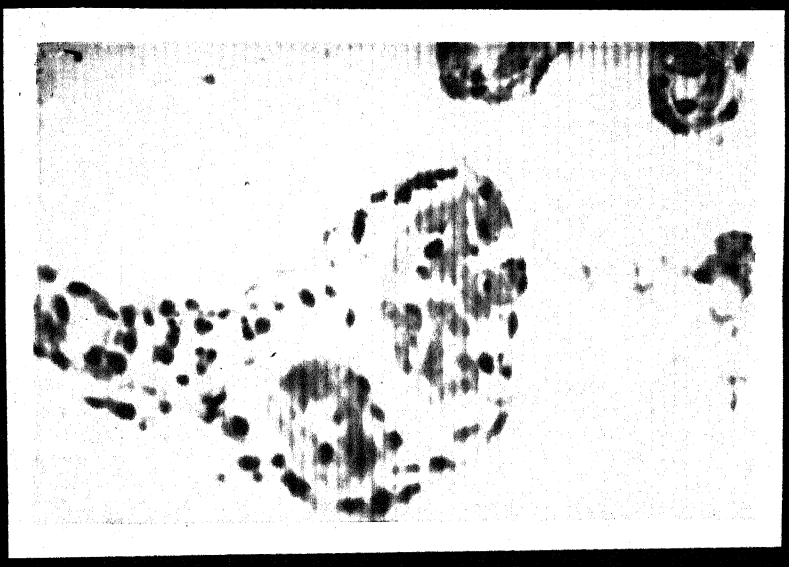
19 Microphotograph of rat heart 7 days after 10mg/kg i.p.i. of Doxorubicin showing large intra myocardial haemorrhage in subepicardial zone. H & E x 280.



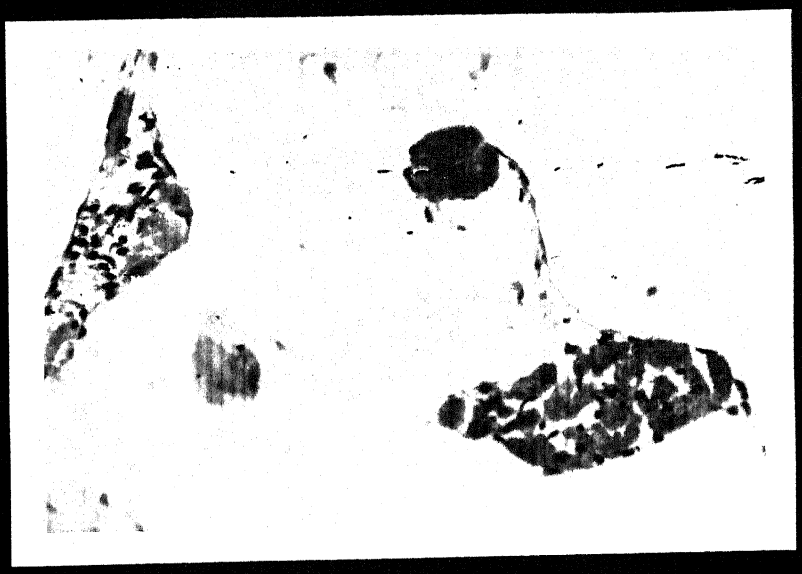
20 Microphotograph of rat heart 14days after 1 mg/kg i.p.i.of Doxorubicin for 10 consecutive days showing dilated lymphatic in the myocardium. H & E x 280.



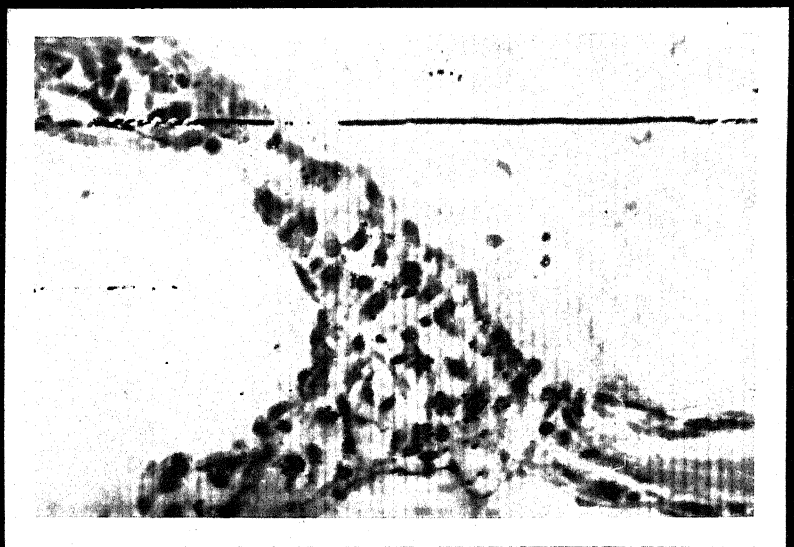
21 Microphotograph of rat heart 3 days after 1 mg/kg i.p.i. of Doxorubicin for 10 consecutive days showing early lesion of myofibre necrosis in a papillary muscle H&E x 280.



12 Microphotograph of rat heart 3 days after 1 mg/kg i.p.i. of Doxorubicin for 10 consecutive days showing an advances lesion of myofibre necrosis & inflammatory reaction in papillary muscle. H & E x 280.

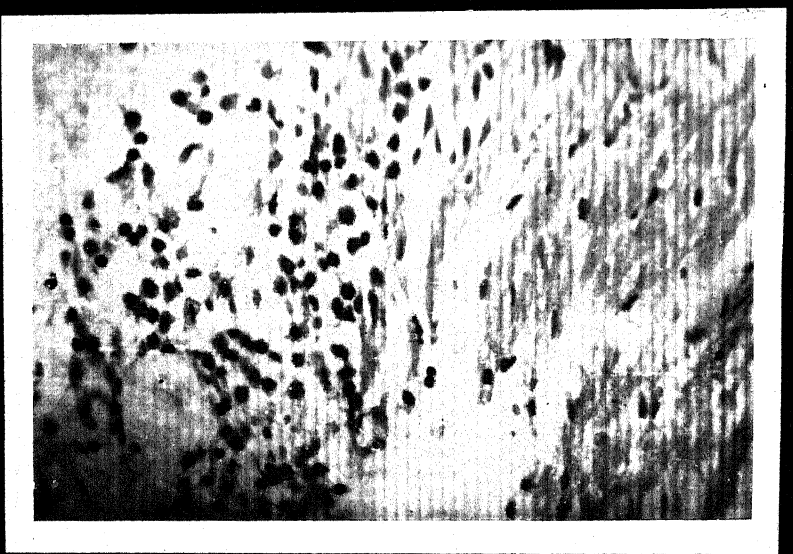


23 Microphotograph of rat heart 3 days after 1 mg/kg i.p.i. of Doxorubicin for 10 consecutive days showing an advanced lesion of myofibre necro-sis and inflammatory reaction in papillary muscle. H & E x 280.



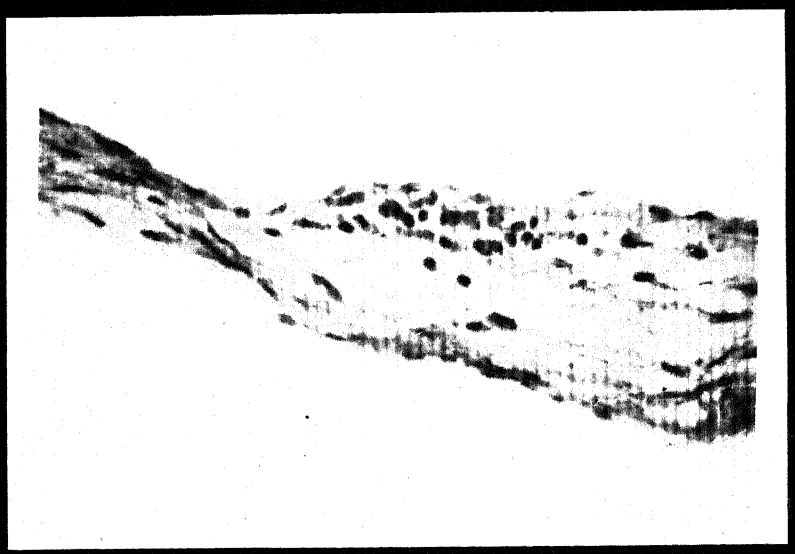
24 Microphotograph of rat heart 3 days after 1 mg/kg i.p.i. of Doxorubicin for 10 consecutive days showing an advanced lesion of myofibre necrosis and inflammatory reaction in papillary muscle. H & E x 280.

25 Microphotograph of rat heart 3 days after 1 mg/kg i.p.i. of Doxorubicin for 10 consecutive days showing an advanced lesion of myofibre necrosis & inflammatory reaction in papillary muscle. H & E x 280.



26 Microphotograph of rat heart 7 days after 5 mg/kg i.p.i. of Doxorubicin on 2 consecutive days showing pericarditis and involvement of sub epicardial zone of myocardium.

27 Microphotograph of rat heart 7 days after 5 mg/kg i.p.i. of Doxorubicin on 2 consecutive days showing valvulitis with involvement of valve ring. H & E x 280.



28 Microphotograph of rat heart 7 days after 5 mg/kg i.p.i. of Doxorubicin on 2 consecutive days showing valvulitis. H & E x 280.

D I S C U S S I O N

DISCUSSION

The anthracycline antibiotics are important antitumour agents. These drugs are sufficiently efficacious to allow for the expression of chronic toxicity. The chronic toxicity that appears to be unique to this class of compounds is a dose related cardiomyopathy (Bristow et al, 1978, Lefark et al, 1973) which limits the use of doxorubicin (Adriamycin) and other anthracyclines.

In addition to chronic toxicity, the anthracycline may also produce acute and subacute cardiovascular effects (Bristow et al, 1978). The acute effects were originally considered to be unrelated to the chronic, as they seemed to have no relationship to the subsequent development of heart failure. However, work carried out by Bristow et al, (1981) in which a dose of doxorubicin, that was associated with the development of a cardiomyopathy, produced acute haemodynamic effects that were entirely related to the release of vasoactive substances, and no acute cardiac effects of doxorubicin were noted in the presence of agents that antagonize histamine and catecholamines. Taylor (1982) in order to assess acute adriamycin cardiotoxicity perfused rabbit hearts with oxygenated krebs-Ringer-bicarbonate buffer at 39^{o}C containing adriamycin for periods of 30 to 150 minutes to determine the very early changes in subcellular structures.

The study done by Olson et al (1977) revealed the subacute cardiotoxicity of adriamycin in rats. The cardiotoxicity was precipitated by single or devided high doses of adriamycin. Adriamycin at dosages of 20 mg per kg, 10 mg per kg X 2, 13 mg per kg, and 10 mg per kg produced fatality, rapid weight loss, fine structural alterations compatible with severe myocardial damage, and significant increases of myocardial tissue calcium and serum enzymes (CPK, LDH).

Clinical and experimental investigations of adriamycin cardiotoxicity has been the delayed dose dependent degenerative cardiomyopathy. Bristow et al (1978) ; Singer et al (1978); Chalcroft et al (1973) and Herman et al (1969) have worked on acute toxicity, and have described fibrinous pericarditis myocarditis, left ventricular failure and arrhythmias as an early manifestation of adriamycin administration.

The purpose of the present investigation was to study the acute effects of adriamycin on rat heart and also to assess the possible mechanism of its cardiotoxicity.

In the present study, myocardial degenerative and necrotic changes were observed in rats following administration of doxorubicin. The lesions commonly consisted of focal areas of myofibre necrosis of variable grades and

inflammatory reaction with lymphocytes and occasional polymorphonuclear leucocytes around areas of myofibre necrosis and small blood vessels. When the cardiac lesions were more pronounced, larger number of myofibres were necrosed, occasionally in confluent areas. Pericarditis and valvulitis with or without involvement of the valve ring were seen in many animals. Cellular oedema with separation of myofibres were observed in the heart of few rats. In most of the cases blood vessels were dilated, few of them were congested and few ruptured with extravasation of blood. Interstitial haemorrhages were a consistent feature; there were numerous small multiple foci of interstitial haemorrhages in most of the animals with large confluent foci of haemorrhage seen at places in few of them.

It was of interest to observe the involvement of papillary muscle in some of the animals which attains special significance as this type of injury may account for early onset of cardiotoxic manifestations resulting in various types of cardiac dysfunctions viz arrhythmias , electrocardiographic changes and the haemodynamic derangements leading to heart failure.

In animals of Group A and B, who received doxorubicin either as a single i.p.i. of 10 mg/kg or as two i.p.i. on two consecutive days of 5 mg/kg each, the histopathological

changes were most marked at day 3 and 7 after the injection and the lesions tended to regress in animals sacrificed on day fourteen. Whereas in animals of Group C who received 10 i.p.i. of 1 mg/kg doxorubicin on 10 consecutive days, the extent and severity of cardiac lesions was almost similar at different period of sacrifice, indicating thereby that repeated doses of doxorubicin, even though small are sufficient to cause persistant myocardial damage in these animals. Obviously these findings have direct significance in clinical practice.

Maral et al (1967) using doxorubicin in doses of 1 mg/kg/day have also reported myocardial lesions consisting of interstitial oedema, degeneration of myocardial fibres and myocytolysis. Farmer et al (1970) using doxorubicin in doses of 8-100 mg/kg single i.p.i. or multiple i.p.i. of 1-10 mg/kg for 4 days have also observed the cardiac changes in the form of dilated and congested myocardial capillaries, ischaemic necrosis of cardiac fibres and slight neutrophilic infiltrations. Lefark et al (1973) studied the cardiotoxic effects of adriamycin in 399 patients treated for advanced carcinoma, the postmortem examination of the hearts in two cases who expired during the treatment with doxorubicin revealed decrease in the number of cardiac muscle cell and degeneration of the myocardial cells.

Although the present study is restricted to the light microscopic changes. Chalcrot (1973) performed studies on fine structural effects of daunorubicin in both atrial and ventricular myocardium. He demonstrated that on 5th day after the single dose of 25 mg daunorubicin/kg i.v.i., along with degenerating mitochondria and membranous whorls resembling myelin figures the myocardium also showed many endothelial cells which were pale and swollen and they frequently bulged into the capillary lumen. These observations support the occurance of vascular dilatation, congestion and rupture of small myocardiac capillaries with extravasation of blood as observed in the present study.

Billingham et al (1978) making use of the percutaneous transvenous endomyocardial biopsy found histopathological lesions to be both focal and disseminated ; their findings of advanced degenerative changes seen isolated against a background of morphologically intact myocardium and the subendocardium representing a particularly prominent site of involvement. These authors also reported vacuolar degeneration of myocytes. Similar observations have also been made in rats treated with doxorubicin in the present study. The electron microscopic studies carried out by these authors have revealed that the earliest manifestation of doxorubicin cardiotoxicity is distension of sarcoplasmic

reticulum which eventually swells and coalesces to form large membrane bound clear spaces in the cytoplasm, these lesions eventually progress until the death of the myocyte at which time the mitochondria do degenerate by swelling and crystolysis, myelin figures appear and the nuclei become pyknotic and disintegrate. However in this study, the endothelial cells of the capillaries and small arterioles were found to be unaffected while in the present study damage to the endothelium resulting into extravasation of the blood was more or less a uniform feature. Bertazzoli et al (1979) reported vacuolization, myolysis myofiberatrophy, thickening of the endocardium, swollen endothelial cells and inflammatory reaction.

There is paucity of work on acute histological changes of cardiotoxicity in animals treated with doxorubicin. However, the histological findings of acute cardiotoxicity of this drug as seen by Taylor et al (1982) are quite similar to those observed in the present study. These authors perfused rabbit hearts with the pefusate having adriamycin at concentrations of either 1, 2 or 8 mg/litre over a period of 30 to 150 minutes. Hearts treated with doxorubicin revealed separation of myofibres consistent with interstitial edema, frequent and marked cytoplasmic vacuolization particularly perinuclear vacuolization in 17/18 hearts showing scattered vacuolization

of myocardial cells and nine of 18 (50 percent) showing marked alteration with some vacuolization evident in virtually every low power field studied. These findings are almost similar to those reported in the present study. Taylor et al (1982) needs emphasis because it gives clue to the histological findings seen by us on light microscopy after giving doxorubicin and taking sections on 1st, 3rd, 7th and 14th day of the drug administration. Taylor et al (1982) studied electron microscopy findings in the myocardial of these animals and observed that the morphologic changes in hearts exposed to adriamycin consisted of disruption of sarcomeres with myocytolysis, cytoplasmic vacuolization and swelling of mitochondria with occasional presence of flocculent densities. A distinctive, central clumping of the nuclear material with clearing of chromatin along the nuclear membrane was evident in the nuclei of all cells from adriamycin exposed hearts. In no instance was the central nuclear clumping with clearing of chromatin from the nuclear membrane occuring in the adriamycin hearts seen in the control hearts. These findings indicate that in acute studies the nucleus is the first organelle to be affected with the cytoplasm frequently minimally altered. Nuclear alterations have also been reported by Jaenke et al (1974); Lambertenghi Deliliers et al (1976); Buja et al (1974).

Acute cardiotoxic manifestations of this drug have also been reported in man. Starkebaum (1975) and Harrison (1976) have independently described a patient in whom pericarditis, myocarditis, fatal pump failure and cardiac arrest developed nine days after treatment with daunorubicin given as three daily doses of 70 mg/m^2.

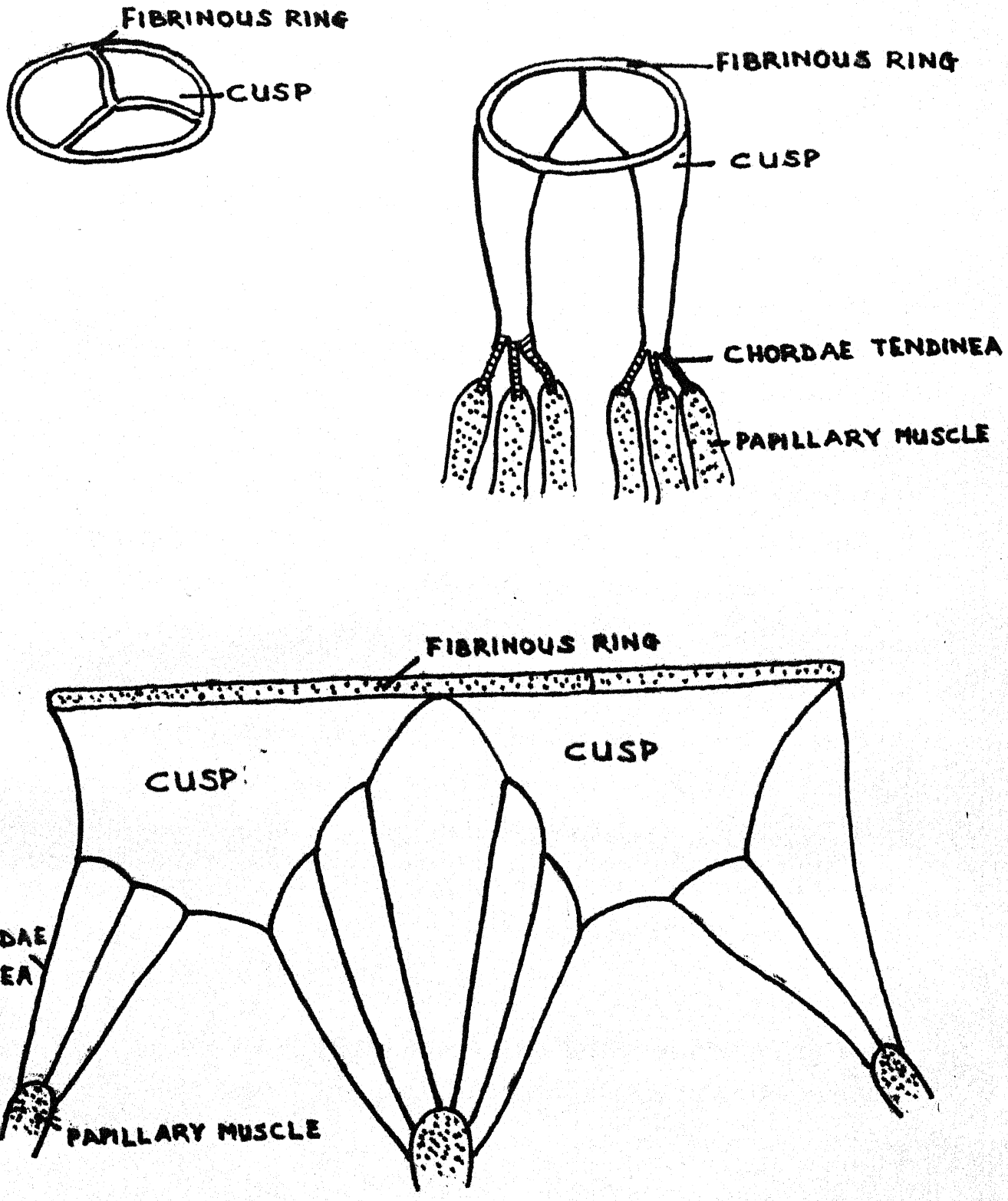
Ippolité (1976) reported the development of cardiogenic shock refractory to therapy in 56 year old man one week after he received 80 mg total dose of daunorubicin, the same investigators reported a case of apparent myocardial infarction 48 hours after the patient received 50 mg dose of daunorubicin. Ainger et al (1971) reported a case of fatal pump failure in a 12 years old child 12 days after the drug administration, shortly before death, the patients electrocardiogram showed an anterior injury pattern. Rinehart et al (1974), Greco et al (1976) and Singer et al (1978) have also described transient deterioration in left ventricular function following doses of doxorubicin.

None of the studies made so far on acute adriamycin cardiotoxicity have mentioned about valvulitis and papillary muscle involvement as observed in the present study in the doxorubucin administered rats. These observation are of considerable clinical interest in view of various

types of acute cardiac dysfunctions reported by various workers in patients receiving doxorubicin even for short periods (Ippolito, 1976; Ainger et al, 1971; Rinehart et al, 1974; Greco et al, 1976; Singer et al, 1978).

Bristow et al (1978) observed early clinically significant cardiotoxic manifestations developed within one to twenty three days of anthracycline administration. They concluded that single doses or initial courses of anthracycline may be associated with atleast three types of cardiac effects. The first is a pericarditis/myocarditis syndrome, which appears to be associated with high mortality and significant impairment of myocardial performance. The second type of early cardiotoxicity is a transient, reversible myocardial dysfunction that may result in reversible heart failure in patients with borderline cardiac reserve. Rhythm disturbances are a third type of early cardiotoxic effect.

The involvement of papillary muscle and valves with cusp damage as seen in the present study, to some extent may explain the early clinically significant cardiotoxic manifestations. In order to know the type of valvular damage, valve ring involvement and lesions affecting the papillary muscles, their anatomical considerations shall help in assessment of possible relationship between structural cardiac damage and cardiac dysfunction which has a direct bearing in clinical assessment of patients on doxorubicin therapy.



ANATOMY OF VALVULAR CUSP CHORDAE TENDINEA AND PAPILLARY MUSCLE

The cardiac valves are made up of fibrinous ring to which the cusps are attached; the cusps are flat and project into the ventricular cavity. Each cusp has an attached and a free margin, and an atrial and ventricular surface. The atrial surface is smooth, the free margins and ventricular surfaces are rough and irregular due to attachement of chordae tendinea. The valves are closed during ventricular systole by apposition of the atrial surfaces near the serrated margins. The chrodae tendinea connect the free margin and ventricular surfaces of the cusps to the apices of the papillary muscle. They prevent eversion of the free margins and limit the amount of balloning of the cusps towards the cavity of the atrium. The atrio ventricular valves are kept competent by active contraction of the papillary muscle, which pull on the chrodae tendinae during ventricular systole. The involvement of papillary muscles along with valve cusps and valve ring cardiotoxicity may cause incompetent atrioventricular valve leading to accumalation of blood in the heart and venous circulation at the expense of arterial volume and ultimately leads to heart failure.

The rhythmic contraction of the heart is called the heart beat. The impulse to contract is generated in specialized, Nodal tissue in the wall of the right atrium.

Impulses are discharged rhythmically from this Sino-Atrial-Node (The "Pacemaker"). The wave of excitation spreads throughout the muscle of both atria which are excited to contract. The impulse is picked up by another mass of Nodal tissue the atrio ventricular node and relayed by Purkinje Tissue (in Bundle of His and its branches) lying beneath the endocardium on the interventricular septum. This relays the impulse to contract to the muscle of both ventricles.

Not infrequent involvement of papillary muscles and valve ring close to A.V. junction as observed in the present study, may hamper the A.V. node function resulting in the causation of arrhythmias and other cardiac dysfunctions, so common in the early anthracycline cardiotoxicity.

Another interesting observation encountered in this study was the demonstrating of contraction band necrosis in the heart of some of the animals. These lesions constitute some what transluscent intensily eosinophilic transverse bands within a myocyte or group of adjoining myocytes. Electron microscopic studies have revealed that these lesions are usually located close to an inter calated disc accompanied by shortening and scalloping of the sarcomeres, fregmentation of the Z band, distortion of the myofilaments, desplacement of the mitochondria away from the intercalated disc. Such a

phenomenon is supposed to have been produced by hyper-contraction of myofibrils in the dying cells consequent to acute transient myocardial ischaemia. Vascular damage as observed in the present study supports such a concept. Whether contrature band myocardial necrosis could play any significant functional cardiac alterations is not known.

In the present study, in order to have some quantitative assessment of myocardial injury in different rats, the attempt was made for numerical scoring of the injury. For this purpose the points awarded to a lesion on half point scale were added and their sum total represented the injury score for the particular animal, based on which a injury score for a batch or a group were calculated which reflected the total injury occuring to myocardium after doxorubicin administration in various doses, and it appeared relatively a better procedure to assess the cardiotoxic damage produced in these animals. Bertazzoli et al (1979) to acess the median cumulative cardiotoxic doses have also used similar quantification.

Despite extensive investigations the precise pathogenetic mechanisms of adriamycin induced cardiotoxicity remain to be defined. Several theories regarding the genesis of adriamycin cardiotoxicity have been advanced. These include release of histamine and catecholamines with resultant myocardial damage; free radical generation and

subsequent lipid peroxidation; effects on various membrane systmes, including $Na^+ - K^+$ ATpase pump; binding of adriamycin to cell membrane lipids; damage to mitochondria; excess calcium influx; and effects on nucleic acids and on protein synthesis. Whatever be the exact mechanism at subcellular level, the histologic picture observed after the drug administration strongly suggest that doxorubicin cardiotoxicity may be initiated by toxic endothelial damage. The endothelial damage leads to capillary microthrombosis and increased permeability of endothelium with leakage of the plasma proteins and erythrocytes into the myocardial interstitium and into myocardial muscule cells themselves. The direct endothelial damage is followed by extravasation of blood containing high levels of doxorubicin with resultant toxic damage of muscle cells. Such a hypothesis of toxic damage of muscle cells has also been reported by D'Agastino (1963).

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSIONS

The anthracycline antibiotics are an effective group of chemotherapeutic agents for use against a wide range of solid and hematologic malignancies. Their use is limited, however, by cardiac toxicity. The precise mechanisms of cardiac toxicity have not yet been identified. Because the anthracyclines have multiple biologic effects including alterations of vasomotor tone and interactions with membrane lipids and with membrane and mitochondrial enzyme system, there are numerous potential mechanisms of damage. The emphasis of most clinical and experimental investigations of adriamycin cardiotoxicity has been the delayed dose dependent degenerative cardiomyopathy. An acute toxicity, however, manifested by fibrinous pericarditis left ventricular failure and arrhythmias has been described in some patients treated with anthracyclines and in animal models. The purpose of the present investigation was to study the acute effects of adriamycin on the heart of rats and to assess the probable mechanism leading to the cardiac injury.

The present work comprised of an experimental study of cardiotoxicity, using doxorubicin. The cardiac injury was produced by the intraperitoneal injections

of doxorubicin in various dosage schedules. The experiments were performed on 42 healthy swiss albino rats. They were divided into 3 groups (A, B & C) of 12 animals each, 6 untreated animals served as healthy control. The salient features of this study are as follows :

GROUP 'A' :

Group A animals were administered with single i.p.i. of 10 mg/kg of doxorubicin. In all the animals there were dilated and congested blood vessels few of them showing leakage and extravasation of blood. The vascular changes were more marked in the subepicardial zone. Out of eleven animals three showed papillary muscle involvement. Most of the animals (9/11) showed multiple small areas of haemorrhages while two rats sacrificed on day 1 and 7 respectively showed large areas of interstitial haemorrhage. Pericarditis and valvulitis with or without involvement of the valve ring was encountered in 7 out of 11 animals. The intensity of myocardial injury gradually increased as the animals sacrificed on day one, showed only cytoplasmic vacuolization whereas those sacrificed at day 3,4, 7 had myofibre necrosis although the severity of cardiac injury regressed at day 14 after the administration of the drug however,occasional foci of myofibre necrosis were still seen in these animals.

GROUP 'B' :

Animals in this group received 5 mg/kg i.p.i. of doxorubicin for 2 consecutive days. In all the animals there were some dilated and congested blood vessels, few of them showed leakage with extravasation of blood, these vascular changes as in the previous group were more pronounced in the subepicardial zone. Out of 12 animals in this group 5 showed papillary muscle involvement. The extent of myofibre necrosis was reduced in animals sacrificed later but the extent of inflammatory reaction showed little change. Most of the animal (9/12) showed multiple small areas of interstitial haemorrhage while one rat sacrificed at day fourteen after the administration of the drug showed large areas of interstitial haemorrhage. Pericarditis and valvulitis was seen in ten out of twelve animals.

GROUP 'C' :

Animals in this group received doxorubicin in doses of 1 mg/kg i.p.i. for ten consecutive days. All the animals in this group showed mild degree of myofibre necrosis. Of the twelve animals four showed moderate degree of inflammatory reaction while eight showed mild inflammation. Pericarditis was seen in nine animals and valvulitis was observed

in most of the animals (11/12). Six animals showed papillary muscle involvement. Small and large foci of haemorrhage were seen as in previous groups. The blood vessels were dilated with or without congestion and rupture.

Contraction band necrosis was a frequent feature in the animals of group A, where (4/11) animals exhibited the contraction band necrosis. Only single animal in group B while none in group C showed contraction band necrosis. The injury score data reveals that the intensity of myocardial injury in group A and B was almost similar while group C animals had the most pronounced cardiac injury.

It is believed that doxorubicin causes direct endothelial damage with extravasation of blood; this leakage of the drug in the myocardium causing myofibre necrosis. The type of valvular damage, valve ring involvement, and lesions involving the papillary muscle shall help in assessment of possible relationship between structural damage and cardiac dysfunction which has a direct bearing in clinical assessment of patients on doxorubicin therapy.

B I B L I O G R A P H Y

B I B L I O G R A P H Y

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